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ON ENDOCRINES AND ORGANISMS

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I

Probably there is more than one reason for the relative success and supremacy of vertebrate animals, three classes of which—fishes, reptiles, mammals—have in succession dominated the animal life of the earth. Among the reasons hitherto suggested, it seems doubtful whether there is anything so important as the extraordinary development in this group of the means of regulation and coordination of the parts of the organism. The nervous system is of outstanding importance in this respect, and its very marked development has often been cited as the thing which has brought strength and distinction to the vertebrate phylum, and particularly to man. In the work of coordination and regulation, however, it has become clear during the last three decades that the endocrine glands also have a large share. It is further becoming evident that the higher invertebrate phyla, though provided with quite complex nervous systems and sense organs, are probably equipped with partial, meager, or with peculiar, endocrine systems. At the outset of this discussion it is therefore fairly evident that not merely a neural, but a nervous-endocrine, development chiefly distinguishes the vertebrates as a group.

The nervous and endocrine systems are not only largely engaged in a common service, but they are probably peculiarly related to each other. This applies in rather

special degree to the autonomic (sympathetic) nervous system. Although our knowledge on this large subject is quite incomplete it is recognized that the amount of some of the incretions is partly regulated by the nervous system and that parts of the nervous system are in turn affected by the altered quantities of these incretions. Again, changes in the activity of one endocrine gland tend to bring about changes in several others; and in the higher vertebrates at least it seems probable that the mid-brain mediates in the adjustment of these changes. Further, sympathetic nerve action falls into stimulatory and inhibitory groups; recognized authorities have made tentative division of the action of the various incretions into stimulatory and inhibitory groups. In any general view of the internal secretions their connection with the nervous system is found to be closer than with other systems of the body (the blood and vascular system not considered). This close functional relationship of the nervous and endocrine systems is stressed in these introductory remarks because the question of a further genetic, or phylogenetic, association of these two systems will later appear as an important element in the present discussion.

Most of current interest in the incretory glands is centered upon the relation they bear to normality and disease, particularly in the human. Their relation to growth and to tissue differentiation are points of interest to an increasing group of workers. Still another group of investigators strive for a fuller understanding of the function and interrelationships of the products of these organs. This indeed represents a wide range of interest in these incretions at the points where their activity touches upon human health, happiness and welfare, and doubtless these very practical inquiries cover the aspects of endocrinology which are of most importance to us as human beings. It is, however, quite another aspect of the endocrine problem that is treated here. Our purpose is to consider the place which the organs of

internal secretion occupy in the evolution of animals—what their presence means—why so many—how their puzzling interrelationships and origin may be pictured in a rational way. Though these questions may not be of immediate practical importance, they involve the luxury of an attempt at a broad and long view of the incretory organs. And, if the solution of some of these questions can be resolved, or even slightly advanced, probably we may then find ourselves in a better position to deal with some practical questions of wide current interest.

Our own studies on reproduction and sex in birds have shown that these functions have hitherto unrecognized relationships with some of the incretory organs. Also, we have obtained some evidence that certain reproductive processes occur quite independently of the nervous system. We have tried to fit these few facts obtained in our own sphere of study to the very large body of facts now known. As a result we are led to the suggestion that most, perhaps all, of the incretions have arisen to regulate processes which nervous action had proved incapable of regulating—namely, *irregularly rhythmic processes;* and that probably the chief of such rhythms, in vertebrates at least, are those involved in *reproduction.* Examination of present knowledge further leads us to suggest that, though "chemical regulation" is phylogenetically quite old, regulation by true hormones is more recent than nervous action and has arisen only or largely after the formation of both central and sympathetic nervous systems in the animal series. On this last-named point our interpretation of the facts distinctly differs from that currently taught. These points and views are developed in the following pages.

II

When either the trained biologist or the layman accomplishes a detached view of the human species, as an animal species, his *first* reaction is perhaps that of self-congratulation at its complexity and finish of structure,

power and equipment. His *second* thought will surely be caught and fixed upon the dimorphism of form and the division of function into two distinct groups of individuals within the species—an ever-present and far-reaching divergence of the sexes. When attention is similarly directed to any other of the thousands of species—any except the lowliest organisms—this second impression dealing with sex-differences will remain; and, reflection upon the fact will bring conviction that sex and sexual reproduction have long had very firm footing in the evolutional marches which led to the origin and survival of the animal world as it now exists.

The first point of fact which I want to examine—and, if possible, to establish—is that new data place an increased number of the incretory organs in a closer relation to reproduction and sex. Since I have personally attempted an intimate study of this problem it is convenient to develop this part of our subject largely on the basis of the work of our own laboratory. During several years our studies have been concerned primarily with processes and mechanisms which seem to govern reproduction and sex. Some problems in this field we think it has been possible to resolve satisfactorily by means of physiological, chemical and breeding studies, and by focusing attention upon the organs primarily concerned in sex and reproduction. But investigation of the most important problems in this field have required the extension of our studies to many or most of the organs of internal secretion; this, because it became clear that the problems of sex and reproduction are almost hopelessly intertwined with the action of a large number of these ductless glands. In our effort to bring some order out of these numerous entanglements between the phenomena of reproduction and sex on the one hand, and the action of the internal secretions on the other, we were forced now several years ago to face the question of the evolutional significance of these complications and entanglements. We can here give our tentative answer to this question.

My earlier studies on sex and reproduction lead me—and I am encouraged to think a growing number of others—to the view that the presence of male and female in animal species generally, including our own species, is in the final analysis accomplished through the establishment of two different rates of metabolism—of two different rates of oxidation and energy release—in the fertilized egg; and in the maintenance of these two different planes or levels of metabolism in the germinal tissues of the developing embryo and adult. Our measurements indicate that the production of normal females is accompanied, in the first or egg-stage, by a lower rate of cellular oxidation, or metabolism, and with higher storage or assimilatory powers; and that the production of males is, in the initial or egg-stage, accompanied by a somewhat higher oxidizing or disassimilatory rate. We regard chromosomal differences of the sexual cells, or better of gametes, as being a usual and normal part of a mechanism to maintain two such different metabolic levels. But it happens that, in addition to the means (genetic factors) which the fertilized egg has for attaining and maintaining these two different levels of metabolism, the organism which develops from such fertilized egg develops within its own growing body a nervous system and a group of organs of internal secretion—both of which probably have a profound influence on the metabolism; and thus, in our view, these several organs must have at least some bearing on sexuality. It should be added that all investigators agree that some of the organs of internal secretion are necessary elements in the *development* of sex in vertebrates. In view of these several facts we have been compelled to consider it impossible to arrive at full and adequate information concerning sex and reproduction apart from searching inquiry on the rôle of the internal secretions.

If sex and metabolism are in fact related in the way just indicated then those incretions which definitely tend to raise or to lower the basal heat production bear this

hitherto unrecognized relation to sexuality. A proper test of such an action can of course be obtained only with the chemically pure or practically pure incretion. Pure thyroxin and adrenin (epinephrin) clearly increase the metabolism. The purification and testing of insulin and the posterior pituitary hormone are only now in process of accomplishment, and the effect of these principles on metabolism is not yet definitely known. Whether any or all of the remaining hormones act on the metabolism is largely a matter of conjecture. It seems probable that the pure incretions of the testis and ovary will also be found to affect the basal metabolism. The incretion of the parathyroid is almost certainly concerned in the regulation of the amount of calcium in the blood.

Quite apart from the above-mentioned relation of the various incretions to sex through their relation to metabolism, the following organs have earlier been described by various investigators as related to *sex:* Thyroid, pituitary, suprarenal cortex, pineal, and of course the testis and ovary. It is not suggested here that these relationships of the endocrines to sex are at present of great medical interest; but if these relationships actually exist they may be of great biological interest.

Turning now to the incretory organs that are usually recognized as being related to *reproduction* we have the following list: Thyroid, anterior and posterior pituitary, suprarenal, testis and ovary. Histological and size changes in these organs occur in mammals either during pregnancy, or lactation, or heat, at puberty or the menopause, or at several of these periods. Still other evidences of changes in the functional level of some of these organs with reference to reproduction seem fairly well established. Working with pigeons we have obtained further and very clear evidence for a relation of most of the above-named organs to reproduction. Here we find that the seasonal size changes in thyroid and gonad (both ovary and testis) are of a reverse or reciprocal order. The thyroids are largest in autumn and winter, when the

gonads are smallest; they are smallest in spring and summer when the gonads are largest. Both cortical and medullary parts of the suprarenals seem to hypertrophy coincident with each ovulation period; and a simultaneous increase in the blood sugar supplies some evidence of a concurrent increase in the output of adrenin. Our evidence, similar to that already known, for the posterior pituitary, testis and ovary does not require notice here.

Other studies in our laboratory have supplied cogent evidence for the participation of still other incretions in essential processes of reproduction. Most important among these is the practical demonstration that an incretion of the thymus (named by us *thymovidin*) is essential to the building of the egg-envelopes (shell, membranes, albumen) in pigeons, and thus is probably similarly necessary in all vertebrates except mammals. Mammals, except the very lowly monotremes, no longer produce these egg-envelopes and so have lost what we conceive to be the original or primary function of the thymus. The discovery of this function of the thymus therefore places this organ prominently among the incretory organs which regulate one or another of the processes of reproduction. Again, it was found that not only does the blood sugar normally increase at ovulation, but that ovulations are usually blocked when this increase is prevented. Thrice daily injections of insulin thus blocked 90 per cent. of all expected ovulations in ring doves. Of course, there exists the possibility that impurities in insulin, rather than the pure hormone, were adverse or harmful to the animal; but the more probable interpretation of the present evidence is that the incretion of the pancreas—through its action on carbohydrate metabolism—is essentially concerned in the primitive type of vertebrate reproduction. Others had earlier found a glycemia in frogs in the period of egg-ripening or growth. Indeed, to whatever extent the anterior pituitary and chromaffin system share in the regulation of these rhythmic increases of blood sugar they also are related to reproduction. Further, we have found

that the parathyroids—as measured by the blood calcium—increase their activity coincident with each ovulation period, the calcium value being more than doubled in pigeons; and this result has been confirmed by others in fowls. Finally, it has been shown both by ourselves and by others that the fat and lipoid phosphorus of the blood of both fowls and pigeons is notably increased at ovulation. The increase of phosphorus values is perhaps in part traceable to parathyroid action; probably the rhythmic lipemia is at least partially of endocrine origin, but the organ or organs primarily concerned can not now be positively identified.

On the other hand, still other studies of our laboratory have indicated that several of the numerous and complicated processes occurring in the bird oviduct are largely—some of them probably completely—independent of the nervous system. The administration of alkaloids (atropine, cocaine, pilocarpine, nicotine) to pigeons has provided evidence that the secretion of shell, shell-membrane, and albumen are all largely or wholly independent of nervous regulation. Even the exact timing of the acts of ovulation and of egg-laying remain unmodified under conditions which presumably greatly modify both sympathetic and parasympathetic action. Nerves are of course present in, and are doubtless necessary to the maintenance of these organs; but these rhythmic functions, together with the phenomenal cyclical growth of all parts of the oviduct, are primarily non-nervous in their origin. The evidence indicates that this cyclical growth is regulated by an incretion of the ovary.

No one can have examined the phenomena of broodiness in birds (particularly the exact timing of the onset of incubation behavior as this affects both sexes in such forms as pigeons), or the occasional profound modification of the crop-glands that occurs only at the end of incubation in birds, or even the migratory performances of many animals, without finding strong indications that the primary basis of these rhythmic processes are non-

nervous and endocrine in character. Evidence that the marked periodic increase of the *vesiculae seminales* in feral birds is of endocrine origin has been obtained (in press).

These studies of our own laboratory thus provide new and additional reason for the view that many or most of the processes of reproduction are under hormonal—not nervous—control; and that this control is shared not only by several of the previously recognized incretory organs (thyroid, testis, ovary, interrenals, pituitary), but by the thymus, pancreas, adrenal medulla and parathyroids in addition. *Thus nearly all the true hormones have now been found to be identified with the processes of reproduction or of sex; many of them indeed with both reproduction and sex.*

III

The processes of reproduction are highly rhythmic and they are restricted to a part only of the span of life. Some of the elements of sexuality are, if not rhythmic, certainly much more emphatic during only parts of the life-term. Puberty and menopause are epochs not only in reproduction, but in sexuality as well. What other essential or primary functions of the organism are similarly limited or circumscribed?

The second point to be considered here is the special relationship existing between the true hormones and rhythmic—particularly *irregularly rhythmic*—activity. At the outset it may not be out of place to recall that even the relatively short and regular rhythms involved in the respiratory and heart movements are only incompletely and rather indirectly under nervous control. The heart muscle of the vertebrate is capable of rhythmic beats in the absence of nerves. Though nerves can here modify the rhythm, either accelerating or retarding heart action, the rhythm itself really rests upon a simple type—simpler than true hormone action—of chemical regulation. The case of the respiratory rhythm is somewhat similar in

that a simple form of chemical regulation is utilized to sustain the nerve action secondarily involved.

On the basis of the facts now known it may well be doubted whether the nervous system of animals often exercises a primary rôle in the regulation of irregular rhythms. And when the irregular rhythms of vertebrate animals are considered the writer at least is much impressed with the extent to which *true hormones* have been brought into action. The figure or chart reproduced here may now be utilized to show this relationship of all the hormones to rhythmic processes, and particularly to those involved in sex and reproduction. This chart brings together many of the facts treated in the present and the immediately preceding section of this paper.

In this chart we seem to attempt a classification of the true hormones. Biedl (1913) and others have well indicated the difficulties of formulating a "true classification of hormones" and it is scarcely asked that our chart be considered as a classification, but rather as a way of getting a look at what we regard as the key to their general functional significance and evolutional origin.

In our diagram we try to convey an idea of the extent to which the true hormones are related to rhythmic phenomena; to what extent to phenomena of sex and reproduction; and to what extent they are related to neither rhythmic nor to reproduction and sex phenomena. Though the divisions of the system are our own, the arrangement of the hormones under the headings used is not made upon our own studies or opinions, but almost wholly upon the work and conclusions of others. In the case of the thymus some slight evidence from the work of others had suggested its relationship as tabulated; it is thought, however, that our own recent study of this organ, as noted above, firmly establishes this relationship from a quite new standpoint.

Our chart usually makes use of the name of the organ producing the hormone in preference to a name of the hormone itself. In cases where there is some or much

doubt that a true hormone is involved the name of the corresponding organ is placed in parentheses. Of course, in the case of many of the hormones placed under the heading, "Considered related to Reproduction or to Sex," the hormone is related also to other quite different things, and very often these other things are considered

CHART I

TRUE HORMONES CLASSIFIED WITH REFERENCE TO RHYTHMIC OR NON-RHYTHMIC PRODUCTION AND TO THEIR RELATION OR LACK OF RELATION TO REPRODUCTION AND SEX

Method of secretion	Unrelated to REPRODUCTION and SEX	Considered related to REPRODUCTION or to SEX	
Rhythmically secreted	(GASTRIN) SECRETIN	(CORPUS LUTEUM) (PLACENTA) (FETAL—for milk secr.)	Mammalian (recent)
Continuously secreted, but fluctuating in amount	ADRENAL (CHROMAFFIN SYS.)* .... PARATHYROID* .... PANCREATIC HORMONE*	OVARY THYROID → POSTERIOR PITUITARY TESTIS → THYMUS ANTERIOR PITUITARY → INTERRENAL (CORTEX) (PINEAL)	

* Our own studies supply evidence that these belong in the group for "Related to Reproduction and Sex."

much the more important. We admit their greater importance to us as individuals—free from care as to the origin of the elements of our origin. They are more important in current physiology and in medicine. But, to students of the origin and evolution of the elements of organisms, it may be that the connection of these organs with the essential species-preserving function of reproduction may go infinitely further in explaining their origin, persistence and development than does, let us say, the comfort and health, the normality and power, that any whole species of animal may enjoy—at some time long after their creation—as the result of the possession of good thyroids, pituitaries and adrenals.

It is thought that the organs tabulated are the only ones which can at present be considered as hormone-producing

organs; probably some that have been included (in parentheses) may prove not to belong here. A glance shows that a fair number of these hormones are themselves rhythmically secreted (secretin, and the more doubtful ones—gastrin, corpus luteum, placenta, fetal). Secretin and gastrin are of course not directly related to sex and reproduction, but their plain and main function is concerned with the irregular rhythms involved in the ingestion and digestion of food. The other three members of this series are not only related to reproduction and sex, but it is of much interest to note that these are phylogenetically the newest of the hormones (mammalian), and that there is here no question of their primary and essential association with reproduction. Though the three hormones listed are questionable, the mammalian ovary probably produces one or more hormones not found in other vertebrates. Remembering that, above all else, the mammal is a higher vertebrate with a *reproductive system* very thoroughly transformed from the ancestral type (still persisting in all other vertebrates) is it merely coincidence that the only new mammalian hormones are unmistakably reproductive hormones? To us this circumstance reinforces other evidence for an impressively large number of true hormones intimately involved in the processes of reproduction and sex.

The list of hormones not rhythmically secreted, but related to reproduction or to sex, is a long one (ovary, thyroid, anterior and posterior pituitary, testis, thymus, interrenal, pineal). This relationship has mostly been shown in mammals. In a previous section I have briefly referred to some of our own evidence that this same relationship is found (no data for the pineal) in a non-mammalian group (birds). The three remaining hormones (adrenal, parathyroid, pancreas), would be classified by others than myself as unrelated to reproduction and sex. We have so placed them in the chart; but, on the basis of our evidence from the bird, briefly reviewed in the previous section, we have indicated (by means of

arrows) that these hormones also may properly belong in the "Reproduction and Sex" column.

At this point it can be noted that in recent years students of the internal secretions and of the autonomic nervous system have usually recognized a fairly close relationship of these two systems to the organs of reproduction. Thus, Langdon Brown (1920) writes:

> The close interaction of the endocrine glands with the sympathetic nervous system on the one hand, and with the reproductive organs on the other, is a fact of paramount importance in the understanding of the emotional response of the sympathetic nervous system, and of the influence of emotion on structure. . . . The sympathetic nervous system, the endocrine glands, and the gonads form a basic tripod entrusted with the duty both of the preservation of the individual and the continuity of the species.

It seems to us that Cannon's (1915) presentation of some aspects of this question provides a fair basis for important parts of the statement just quoted. Again, the sympathetic system, not being under control of the will, being adapted for widespread diffuse effects, and being more subject to endocrine influence, is more intimately associated with reproduction that is the central nervous system.

If the classifications of our chart are correct only secretin and gastrin are without relationship to the irregularly rhythmic processes of reproduction and sex. And these two agents coordinate highly irregularly rhythmic functions. We conclude that the true hormones are at present best regarded as agents devised by higher organisms for the activities and coordinations involved in essential and irregular rhythms. The relative inadequacy of nervous coordination for this type of activity, and the later evolutional appearance of hormones than of nervous structures, will be discussed immediately.

IV

The question of the relative order of appearance, in an evolutional sense, of "chemical regulation," nervous regulation and hormone regulation is the third point to be considered. Or, perhaps it is better to say that this

is a point to be "reconsidered." The currently accepted view was very ably put forward by Starling in 1905:

> In man and the higher animals the marvelous adaptations effected by means of the central nervous system are so much in evidence that physiologists have been tempted to ascribe every nexus between distant organs to the intervention of the nervous system; the more so because by this means an adaptation to changes, internal or external, can be effected in many cases within a fraction of a second. But in the evolution of life upon this earth this method of adaptation is of comparatively late appearance and is confined almost entirely to one division of living beings—*i.e.,* the animal kingdom. In the lowest organisms, the unicellular, such as the bacteria and protozoa, the only adaptations into the mechanism of which we can gain any clear insight are those to the environment of the organism and in these cases the mechanism is almost entirely a chemical one. The organism approaches its food or flies from harmful media in consequence of chemical stimuli; it prepares its food for digestion or digests it by the formation of chemical substances, toxins or enzymes. In the lowest metazoa, such as the sponges, there is still no trace of any nervous system. The coordination between the different cells of the colony is still determined by purely chemical means. The aggregation of the phagocytic cells round a foreign body is apparently due to the attraction exerted on them by the chemical substances produced in the death of the injured tissues.
>
> With the appearance of a central nervous system or systems in the higher metazoa the quick motor reactions determined by this system form the most obvious vital manifestations of the animal. But the nervous system has been evolved for quick adaptations, not for the abolition of the chemical correlations which existed before a nervous system came into being. A study of the phenomena of even the highest animals shows that the development of the quick nervous adaptations involves no abrogation of the other more primitive class of reactions—*i.e.,* the chemical ones. Where the reaction is one occupying seconds or fractions of a second the nervous system is of necessity employed. Where the reaction may take minutes, hours, or even days for its accomplishment the nexus between the organs implicated may be chemical.

At the time Starling wrote neural structures were unknown in the protozoa and in the sponges. It is still possible that such structures do not exist there, though recent studies by Kofoid and others seem to raise some question as to their absence even in the protozoa. It is certain, however, that the coelenterates and lower worms have well developed neural structures, though there is no evidence whatever of the presence of true hormones in any of these lowly forms. Of much significance too is the circumstance that none of the examples of early-appearing "chemical regulation" cited by Starling involves true

"hormone" action (elsewhere, however, he includes secretin). In the light of the advances of knowledge since 1905 it is now clear that the types of chemical regulation cited in our quotation are all simpler than hormone regulation *and these precede true hormone regulation as well as neural regulation.* Indeed, it now appears that *nervous regulation* is more primitive than regulation by true hormones; no true hormones having yet been at all indicated in any forms lower than the annelids. This point is of importance to the whole of the view developed here. We think that the facts now available indicate that the evolutional order of appearance of the various means of coordination is: (a) The simplest forms (non-hormone) of chemical regulation; (b) neural regulation; (c) hormone regulation.

According to Starling nervous regulation arose later than chemical adjustments (with these not sub-divided into "simple chemical" and "true hormone" action); and, in effect, this neural development added to the existing *slow* reactions other reactions much more *rapid.* Confining ourselves to hormone action, it seems to us that we may now say that this type of chemical coordination appeared only after the development of a nervous system—central and probably also autonomic—and that the usefulness and effectiveness of hormone action does not rest on a question of slow or rapid reactivity, but in its supplementing the nervous system which had proved ineffective or inadequate in regulating one large group of activities—namely, *irregularly rhythmic* functions. Mammals possess not only the most complicated of nervous systems, but probably also the most complicated of endocrine systems.

We may next consider some facts bearing on the order of origin of neural and hormonal regulation in the animal series. At the outset we may note that if it turns out that true hormones exist in the plant kingdom (rather near physiological equivalents of the adrenal, pancreatic and ovarian hormones have been reported) it can then be

said that the highest as well as the lowest types of chemical regulation have evolved before or apart from any development of neural structures. Among animals, however, there is no difficulty in identifying neural structures in some of the simpler phyla in which there is not only no present evidence for the existence of true hormones, but where also there seems little or no reason to suspect their existence. The nature and extent of neural organization in the coelenterates, together with considerations on the basis this affords for more advanced nervous systems, are questions treated by Parker (1919). But, in contrast with the early appearance of the neural structures, in the tracing of any known hormone backward from higher vertebrates all evidence of its presence is lost before the four or five lower phyla are reached.

Adrenin (epinephrin, adrenalin) has thus been traced downward in the evolutional series further than any other hormone. But the trail is lost if we pass below the leeches and the crayfish. This case, moreover, involves the chromaffin tissue and this tissue affords especially good evidence that it arose *after* the nervous system; for the facts of comparative anatomy and embryology point strongly to the cells of the chromaffin system—which secrete adrenin—as specialized or transformed nerve cells; indeed, probably transformed from nerve cells of the *sympathetic* system. This latter point is of some significance since there is some reason for believing that the sympathetic is of more recent development than the central nervous system. Gaskell's (1916) description of the origin of the sympathetic and chromaffin cells in a particular case pictures the two as moving out together from the central nervous system. The chromaffin cell is not there considered by him as a derivative of sympathetic cells, but I believe it is now thus commonly regarded. Thus, so far as this is known, the hormone that can be traced farthest into ancestral forms discloses its origin in a *neural*—probably a relatively *recent* neural—structure.

It is of interest also that the posterior pituitary and the pineal are of nervous origin. The attempts to identify the homologues of these organs in higher invertebrates have most often hit upon a neural structure. Some worthy evidence for such an origin could be cited. So far as I am aware the thyroid is the only other hormone-producing vertebrate organ which it has been thought possible to trace into present invertebrate forms. In this case Gaskell saw some reason for identifying it as primitively a uterus—a *reproductive* organ. Except for the undoubted nervous nature or derivation of the posterior pituitary and pineal, these matters are of course highly speculative.

The studies of numerous investigators show that the lowest, or at any rate very low, vertebrates are provided with thyroids, thymus, adrenals, interrenals, pancreas, pineals, pituitary, interstitial testis and interstitial ovary, and probably with parathyroids. Surely the chromaffin tissues, and possibly the homologues of the thyroid, pineal and pituitary, have also been found in some of the higher invertebrates. With the exception of the first-named, however, the homologous invertebrate organs apparently perform in these animals a function other than hormone production. It is, of course, to be granted that real difficulties attend all attempts to isolate true hormones in such small animals as insects and worms. Possibly true hormones exist and have escaped discovery in these and still lower forms. But it is an interesting circumstance that *testis* and *ovary* are readily identifiable even down to relatively *low* forms of invertebrate life. An awkward limitation of this fact, however, rests in the great uncertainty as to the presence of an *interstitial* testis or *interstitial* ovary (*i.e.,* the allegedly endocrine portion of the gland) in any—even the highest—of the invertebrata. Such tissue does not appear to exist there. But in the case of the ovary at least this difficulty would partly disappear if—as a few workers think true in vertebrates—it should turn out that the important ovarian

hormone is produced in the ovum itself or in the follicular cells which immediately surround it. Surely a primitive form of the ovary and testis exists in very low invertebrates, though evidence for an associated hormone is almost or quite lacking. The point is raised here because in the fact of the early origin of a reproductive testis and ovary lies the possibility that the internal secretion *first* evolved was one primarily concerned in sex and reproduction.

It should now be noted that in the higher Invertebrata, where there is no or scant evidence for the existence of more than one hormone, there exist fairly well-developed *sympathetic* nervous systems. This is true in the Arthropoda (Insecta, Crustacea), Echinodermata and Coelhelminthes. Present knowledge, therefore, tends to support the view that in the animal series true hormone production was preceded not only by a central nervous system but by a sympathetic or autonomic system as well.

Since considerable stress is laid on the rôle of irregular rhythms as a special condition for the need and for the origin of true hormones in complex animals, it may avoid misunderstanding to state that the writer does not forget that rhythm, in some or many forms, exists in the simplest organisms—wherever there is life. In bacteria and the simplest protozoa cell-division (reproduction) may be regarded as rhythmic. In other protozoa and simple metazoa still other rhythms are well known. These small and simplest organisms, however, seem to have proved their capacity to effect these rhythms and all else that they perform without either neural or hormonal control. It was advance in differentiation, size and structure that required the development of coordinating agents, and what we stress is the indication that hormonal control has thus proved its special effectiveness in the case of highly irregular rhythms in large and complex animals. The lines of demarcation are not regarded as entirely fixed and precise; for example, in the case of pendular movements of the intestine nerve action does not appear to be involved, though it may be concerned in peristalsis.

In this connection certain aspects of the evolution of the processes of reproduction require consideration. In the lowest forms reproduction is by the simple process of fission and here it seems possible to grant freely that neither hormone nor nervous action is required; it would be difficult to suppose, however, that simple forms of chemical regulation are absent in the case of any mentionable activity of a lower organism. Probably the same may be said for reproduction in the sponges, coelenterates and some worms where, in addition to the formation of sperm and ova, budding may also occur. The reproductive processes of such forms as the molluscs, echinoderms and arthropods are, however, sufficiently complex to seem to require coordination, if vertebrate reproduction has in fact supplied the primary need for many or most of the long list of hormones that have arisen there. This consideration undoubtedly raises a question of the soundness either of that part of our present view which stresses the primary relation of hormones to reproduction, or to another part which utilizes the paucity or possible absence of hormones in these higher invertebrates as an example of the earlier development of central and sympathetic nervous systems in the animal series. Some difficulties, which we have no intention of minimizing, do exist in this situation and a further word concerning it is necessary.

In the higher invertebrates last-mentioned it is perhaps permissible to suspect that there exist some hormones which have hitherto escaped detection; possibly too these hormones may even be quite different chemically from those performing similar functions in the vertebrates. The divergence of these and the pro-vertebrate branches of the evolutional tree would permit the latter assumption. One has not, however, equal reason to assume that one or another type of true hormone is present in such lowly forms as the coelenterates where reproduction probably requires neither nervous nor hormonal coordination, but where nevertheless the nerve-net is known to

exist. In the higher invertebrates the relation of hormones to reproduction is at present quite unknown. Future studies in this part of the animal series must provide the basis for any valuable view as to the extent that reproduction is there dependent on hormones; and those studies too must finally decide whether or to what extent the sympathetic system preceded the origin of true hormones. From this intermediate zone of the animal world the facts are not now available. Meanwhile, facts from the sphere of the vertebrates and from the lowest invertebrates give rise to and seem to us to support the various general views developed here.

In 1908 Gaskell presented cogent evidence that the nervous system has been the dominant factor in vertebrate evolution, *i.e.*, other systems have been modified and changed in function in order, so to speak, to give opportunity for the growth and consistent progress of this preeminently important one. As Gaskell put it, "The law of progress is this—The race is not to the swift, nor to the strong, but to the wise." The work of Parker and others gave us fair assurance that muscle cells were differentiated in the animal series earlier than nerve cells. Starling was quick to point out that chemical regulation even precedes the differentiation of any tissues whatsoever; and that this type of regulation is almost everywhere in the organism adjutant to the nervous system of higher animals. Our suggestions are: That the special form of chemical regulation exercised by the true hormones is of later development, of more recent origin, than the nervous system—apparently arising after both the voluntary and involuntary nervous systems; that true hormones arose to effect regulations and balances which are inefficiently done or impossible of accomplishment through nervous action alone; that it is, therefore, the nervous-endocrine system which has been the dominant factor in vertebrate evolution; that the inadequacy of the nervous system is exhibited particularly in the regulation of rhythmic phenomena (other than those in

which impulses are frequent); that irregular rhythms were the points of the greatest inadequacy of nervous coordination; and, finally, that the rhythms of reproduction are the oldest, the most extensively endocrine-regulated, and the most essential to the perpetuation of the race, of these irregular rhythms. If then an "endocrine amendment" may be added to Gaskell's dictum it would run: "The law of progress is this—The race is not to the *unregulated* swift, nor to the *nervous* strong, but to the *balanced* wise."

V

Our studies on reproduction and sex indicate that these functions show some hitherto unrecognized relationships to the organs of internal secretion. The numerous true hormones now known or thought to be intimately related to sex and reproduction provide a new reason for inquiry into the special evolutional meaning of the incretory organs.

In a grouping, or provisional classification, of true hormones it is observed that in vertebrates they all bear a notable relationship to irregularly rhythmic processes —particularly to those of reproduction and sex.

A consideration of the relative order of the evolutional origin of the coordinating mechanisms of the body leads to the view that the nervous system, probably both central and sympathetic, arose earlier than the true hormones. Starling's widely accepted views thus require restatement. First came the simple forms (non-hormonal) of chemical regulation; next, neural regulation; last, true hormonal regulation.

It is not merely a nervous, but a nervous-endocrine, development that has enabled the Vertebrata and man to dominate the living world.

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THE EFFECTS OF ALCOHOL ON BIRTH WEIGHT AND LITTER SIZE IN THE ALBINO RAT

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INTRODUCTION

THIS report deals with the effects of alcohol fumes upon birth weight and litter size in the albino rat. It covers ten generations of rats which were subjected daily to the fumes of alcohol in an air-tight tank.

Treatment of the first generation was begun when the animals were sixteen days of age and continued for a period of one year. In the nine succeeding generations treatment commenced at the age of twenty days and was stopped at one hundred days—an age when this animal is sexually mature and the growth curve flattens out to a plateau.

The controls of the first generation were brothers and sisters—litter mates—of the treated. All animals in the experiment are direct descendants of these two groups—controls and treated—of the first generation. Matings throughout were strictly of one type, sister-by-brother within the litter.

Litter size and birth weight were recorded as soon as possible after the delivery of the litter. The colony was visited every morning and all litters recorded that were born during the night preceding. When litters were born during the day records were taken as soon as parturition was completed. From our experience in observing the 482 litters recorded in this experiment, and also many hundreds of litters in other experiments or in our general stock, it seems safe to conclude that the large majority of litters are born during the night and probably many of these toward morning, as more females were found in labor in the morning than at any other time of day.

Each litter was given a litter number when the litter size and birth weight were recorded. The litter size was checked again at twenty days when the second weighing was made. Litter size does not always check twenty days later, for some mortality occurs during the first twenty days of life. In a very few instances a litter had one or more additional animals at twenty days than at birth. This could not be accounted for by pooling of litters, for only one mother and her litter were kept in a cage. Cases of superfetation have been recorded for the rat, and as in these litters of larger number at the later date there were generally one or two runts, superfetation seems to explain the discrepancy. These were distributed about evenly throughout the experiment and in so large a number as 482 litters, comprising 3,041 new-born rats, can have no statistical significance.

The alcoholic treatment was stopped at the end of the tenth generation, but both control and treated lines were allowed to produce the eleventh generation, and data for this generation are included in the tables.

This is one of a series of papers describing various phases of the experiment. Those already published include: Hanson, 1923, 1923a; Hanson and Handy, 1923; Hanson and Heys, 1924, 1924a, 1926, 1927; Hanson and Sholes, 1924.

A more detailed account of the general setting of the experiment than that given above is contained in these earlier papers and reference is made to them for those wishing more information on the methods used.

Tables I to IV give the constants arrived at by the usual biometrical methods, and figure 1 is a graphic representation of the results obtained.

EXPERIMENTAL RESULTS

A. Birth weight.

Table I gives the mean birth weight per rat in grams for each generation of the treated and control and also

TABLE I

A TABLE SHOWING VARIATION CONSTANTS FOR BIRTH WEIGHT IN GRAMS FOR CONTROL AND TREATED RATS OF THE SECOND TO ELEVENTH GENERATIONS INCLUSIVE

	Control			Treated		
Gen.	Mean	S. D.	C. V.	Mean	S. D.	C. V.
2	6.41 ± 0.11 (95)	1.55 ± 0.08	24.18 ± 1.18	7.61 ± 0.39 (26)	2.95 ± 0.28	38.76 ± 3.63
3	6.40 ± 0.21 (81)	2.38 ± 0.12	36.34 ± 1.90	5.18 ± 0.05 (109)	0.80 ± 0.04	15.44 ± 0.71
4	5.22 ± 0.05 (173)	0.91 ± 0.03	17.43 ± 0.63	5.09 ± 0.03 (210)	0.60 ± 0.02	11.86 ± 0.39
5	5.34 ± 0.04 (169)	0.73 ± 0.03	13.70 ± 0.50	5.48 ± 0.45 (272)	1.11 ± 0.32	20.28 ± 0.59
6	5.45 ± 0.06 (170)	1.30 ± 0.05	23.85 ± 0.87	5.05 ± 0.03 (192)	0.60 ± 0.03	11.91 ± 0.42
7	5.14 ± 0.04 (207)	0.75 ± 0.02	14.59 ± 0.48	5.33 ± 0.55 (82)	0.75 ± 0.04	14.07 ± 0.74
8	5.35 ± 0.05 (207)	1.04 ± 0.03	20.16 ± 0.68	5.35 ± 0.04 (241)	0.89 ± 0.03	16.62 ± 0.51
9	5.62 ± 0.08 (115)	1.23 ± 0.05	21.96 ± 0.98	5.69 ± 0.08 (215)	1.18 ± 0.06	20.75 ± 0.67
10	5.43 ± 0.05 (174)	1.25 ± 0.05	23.11 ± 0.84	4.92 ± 0.04 (303)	0.97 ± 0.03	19.64 ± 0.54
Total	5.49 ± 0.02 (1391)	1.18 ± 0.02	21.53 ± 0.28	5.30 ± 0.17 (1650)	1.10 ± 0.01	20.70 ± 0.36
11	6.23 ± 0.06 (175)	1.24 ± 0.04	19.87 ± 0.72	5.81 ± 0.14 (249)	1.03 ± 0.03	17.81 ± 0.54

the mean birth weight for the ten generations combined. Standard deviation and coefficient of variation are also included.

In the second generation mean birth weight is high in both control and test lines. However, the number of animals is small on the treated side of this generation and the constant may not represent what the mean based on a larger number would. Nevertheless, as the experiment was just getting under way at this point and as the control mean is also high it is probably a fair value for this strain of rats at the beginning of the experiment. The difference between these two means divided by its probable error is 2.93, or just approaching significance.

In the third generation the control mean birth weight remains practically unchanged, while a drop to 5.18 grams occurs in the test animals, creating a difference that is clearly significant. But in the fourth generation the control line drops off to a point near that of the test animals and the difference is negligible. This is even more true of the fifth generation, the probable error of the difference being greater than is the difference. And this is also found to be the case in all remaining generations with the exception of the sixth. Here the difference is clearly a significant one. There are, then, out of all the generations compared two that are statistically significant, the third and the sixth.

When the mean birth weight of all rats for all generations of the controls (1,391 animals) is compared with the corresponding constant (1,650 animals) on the treated side, the following statement gives the clear-cut result obtained:

$$\text{Control } 5.49 \pm 0.02 \text{ minus Test } 5.30 \pm 0.17 = 0.19 \pm 0.17.$$

As this difference is only 1.11 times its probable error, it may be safely concluded that in dealing with the mean birth weight of all generations it can not be held that alcohol has decreased the birth weight of the treated rats.

However, it still remains to discuss the fact that in two generations, the third and sixth, there is a significant difference between the two lines of rats. Perhaps the first explanation that occurs to one is that in these two generations the effects of the alcoholic treatment of the parents is discernible in the birth weight of their offspring. It may be that the alcohol is here acting as a selective factor and that at intervals throughout the experiment there may be expected a housecleaning of all inferior germ cells, the result of which would be to restore the normal birth weight in the succeeding generation. In keeping with this theory is the undoubted fact that in the generation (fourth) following the first significant difference the mean birth weight of the treated is only 0.13 ± 0.06 grams less than the corresponding control; and in the generation (seventh) following the second significant difference, the tests are actually, though not significantly, greater than the controls.

Can a deleterious agent, such as alcohol fumes, act periodically in the elimination of weak germ cells, so that a species under strain, at intervals of several generations, produces not from the ordinary mean of all its germ cells, but from those germ cells that have been able to withstand the untoward conditions—thus purifying and strengthening the stock and bringing it back to normal or even, as would be expected on this hypothesis, improving it? Both Pearl (1924) and Stockard (1924) have discussed these possibilities.

If the above theory be of value, one of its weaknesses in this present work must be pointed out, namely, that in the five generations following the sixth the same wide departure of the test animals from the controls did not again occur. Of course it is not impossible that two such drastic selective experiences as those in the third and sixth generations would stabilize the stock for a long period and this is particularly true when it is recalled that the closest form of inbreeding possible for mammals was practiced throughout the entire experiment, thus

tending to conserve any gains accomplished by these two periods of severe selection.

There is an alternative explanation of the unusual results shown in the third and sixth generations which may partially account for them. As will be brought out later on, there is considerable evidence in the literature to show that birth weight and litter size are negatively correlated. Large litters decrease individual birth weight and conversely small litters contain heavier than normal young. The third generation (Table III) shows that the treated litter size was 8.38 rats per litter, by far the largest constant for litter size in any generation among either control or test animals. The small birth weight of the rats in this generation may be nothing more than the reflection of this abnormally large mean litter size. However, this is not so strikingly true of the sixth generation, yet even here the litter size (6.40) is well above the average, and the mean birth weight, while not the smallest recorded, is, nevertheless, low.

There is still a third possible interpretation of these results which has already been indicated. When the individual generations are disregarded and the totals compared it seems safe to conclude that the very insignificant difference of 0.19 ± 0.17 in mean gram birth weight between the two groups, covering several years in time, nine rat generations and over three thousand animals, is clear evidence that on the average the test group has produced as successfully as the control group.

Is birth weight less variable among the offspring of alcoholic rats than it is in the controls? Table II gives the differences between the coefficients of variation for each generation and for the totals of the entire experiment. In the second and fifth generations the tests are more variable, while in all other generations and the totals the tests are less variable than the controls. These differences are for most generations significant ones.

It seems to be true that for the most part litters of alcoholic parentage are more uniform in birth weight than

TABLE II

A Table showing the Differences between the Mean Birth Weight of the Control and Treated Rats. The Differences in Variability as expressed by the Coefficients of Variability are Also given

Gen.	Mean Diff. +, tests more −, tests less	$\frac{\text{Diff.}}{\text{P. E.}}$	C. V. Diff. +, tests more −, tests less	$\frac{\text{Diff.}}{\text{P. E.}}$
2	+ 1.20 ± 0.41	2.93	+ 14.58 ± 3.82	3.82
3	− 1.22 ± 0.21	5.78	− 20.90 ± 2.03	10.29
4	− 0.13 ± 0.06	2.16	− 5.57 ± 0.75	7.46
5	+ 0.14 ± 0.55		+ 6.30 ± 0.77	7.21
6	− 0.40 ± 0.07	5.71	− 11.94 ± 0.97	12.36
7	+ 0.19 ± 0.56		− 0.52 ± 0.88	
8	− 0.00 ± 0.06		− 3.54 ± 0.85	4.16
9	+ 0.07 ± 0.11		− 1.21 ± 0.68	1.79
10	− 0.51 ± 0.55		− 3.47 ± 0.10	3.48
Totals ...	− 0.19 ± 0.17	1.11	− 0.83 ± 0.46	1.82
11	− 0.42 ± 0.15	2.75	− 2.06 ± 0.90	2.30

those derived from non-alcoholic parents. On the supposition above referred to that alcohol is here acting as a selective agent this is in accordance with the expectation. For if germ cells are being eliminated by the alcohol the range of variability from which the treated young are being produced is a more limited one than when such elimination does not take place.

Pearl (1916, 1917) found that there was no significant difference in mean hatching weight between treated and control fowls, except in the case of his group in which both parents were treated. Here there was a clearly significant difference, but it was in favor of the offspring of the alcoholic birds. If hatching weight in birds can be compared with birth weight in mammals, then Pearl's data are in general agreement with ours, that the offspring of the treated either are superior at birth, or at least, not inferior to those of the controls.

Gates (1925) finds that in mice the general average birth weight of males and females is the same. Miller (1911) and King (1915, 1923) give data for birth weight

TABLE III

A TABLE SHOWING VARIATION CONSTANTS FOR LITTER SIZE OF CONTROL AND TREATED RATS OF THE SECOND TO ELEVENTH GENERATIONS INCLUSIVE. THE NUMBERS IN PARENTHESES ARE NUMBERS OF LITTERS, NOT NUMBERS OF RATS

	Control			Treated		
Gen.	Mean	S. D.	C. V.	Mean	S. D.	C. V.
2	6.79 ± 0.45 (14)	2.51 ± 0.32	36.97 ± 4.71	5.20 ± 0.70 (5)	2.32 ± 0.50	44.64 ± 9.52
3	5.00 ± 0.47 (17)	2.87 ± 0.33	57.40 ± 6.64	8.38 ± 0.44 (13)	2.37 ± 0.31	28.28 ± 3.74
4	6.07 ± 0.32 (29)	2.60 ± 0.23	42.83 ± 3.79	6.73 ± 0.23 (33)	2.02 ± 0.17	29.57 ± 2.46
5	6.17 ± 0.31 (29)	2.51 ± 0.22	40.68 ± 3.61	7.40 ± 0.27 (37)	2.47 ± 0.19	33.27 ± 2.61
6	5.65 ± 0.29 (31)	2.39 ± 0.21	42.30 ± 3.63	6.40 ± 0.31 (30)	2.55 ± 0.23	38.52 ± 3.41
7	5.31 ± 0.22 (39)	2.01 ± 0.15	37.85 ± 2.89	5.73 ± 0.52 (15)	2.99 ± 0.37	52.18 ± 6.42
8	5.24 ± 0.19 (41)	1.82 ± 0.02	34.77 ± 0.26	6 31 ± 0.33 (39)	3.04 ± 0.23	48.14 ± 3.68
9	6.38 ± 0.18 (18)	1.16 ± 0.13	18.22 ± 2.06	7.06 ± 0.30 (31)	2.51 ± 0.02	35.60 ± 3.05
10	6.69 ± 0.04 (26)	2.33 ± 0.02	34.87 ± 3.23	7.54 ± 0.32 (35)	2.79 ± 0.23	37.02 ± 2.98
Totals	5.82 ± 0.10 (244)	2.25 ± 0.07	38.58 ± 1.18	6.88 ± 0.11 (238)	2.67 ± 0.08	39.15 ± 1.20
11	5.83 ± 0.26 (30)	2.14 ± 0.19	36.72 ± 3.20	7.47 ± 0.30 (32)	2.50 ± 0.21	33.51 ± 2.82

of males and females in rats, King finding that in the albino rats of the Wistar Institute stock the average birth weight of the males was 4.69 grams and 4.50 grams for the females.

Our data are not composed of separate weighings for the two sexes. The entire litter was weighed and divided by the total number of young in the litter. This gives more accurate results than individual weighings, but has the disadvantage of including both sexes. However, since the average difference in birth weight between males and females in King's data was only 0.19 of a gram, this is not a serious difference in any event, and becomes negligible in our data in view of the large numbers used and the approximate equality of the sexes. Hanson and Heys

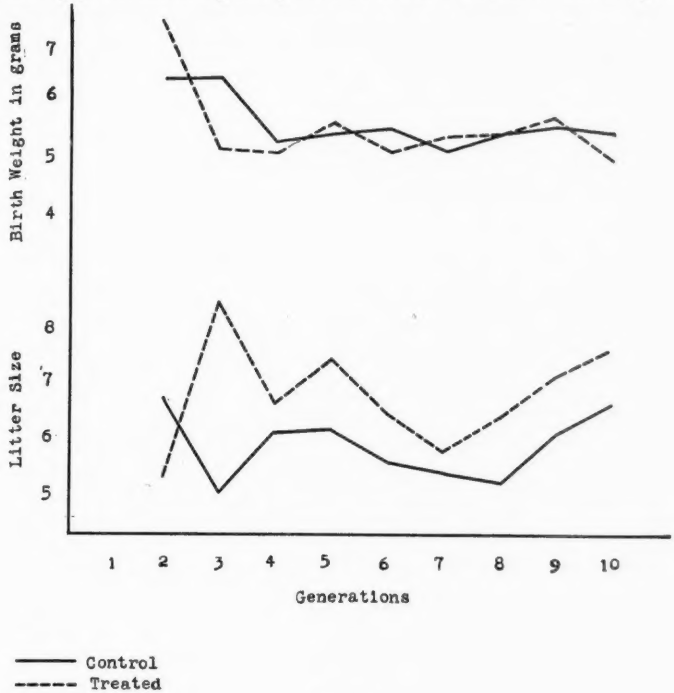

FIG. 1

(1925) have shown that in their rats the sex ratio is 49.41 ± 0.89 (1,435 animals).

B. Litter size.

Tables III and IV and figure 1 give the basic data for litter size in this experiment.

Reference to Table IV reveals an outstanding fact, namely, that in all generations except the second the litter size is larger in the treated than it is in the controls. In four of the constants compared this difference is a statistically valid one, and in two other generations, the eighth and tenth, it is approaching significance. The constants for the one exception to this are in the second generation and here the number of animals, especially on the treated side, is possibly too small to be of value. Omitting this generation from consideration the data for all generations show a difference in favor of the tests that is 6.62 times its probable error—a deviation so great as to be unquestionably valid and presumably due to the only known differential factor, alcohol, in the lives of the control and treated animals.

TABLE IV

A Table showing the Differences between the Mean Litter Size of the Control and Alcoholic Rats. The Differences in Variability as expressed by the Coefficients of Variation are also given

Gen.	Mean Diff. +, tests more −, tests less	$\frac{\text{Diff.}}{\text{P. E.}}$	C. V. Diff. +, tests more −, tests less	$\frac{\text{Diff.}}{\text{P. E.}}$
2	− 1.59 ± 0.83	1.91	+ 7.67 ± 10.62	
3	+ 3.38 ± 0.64	5.28	− 49.12 ± 7.62	6.45
4	+ 0.66 ± 0.39	1.69	− 13.26 ± 4.52	2.94
5	+ 1.23 ± 0.41	3.00	− 7.41 ± 4.45	1.67
6	+ 0.75 ± 0.43	1.78	− 3.78 ± 4.98	
7	+ 0.42 ± 0.56		+ 14.33 ± 7.04	2.04
8	+ 1.07 ± 0.38	2.82	+ 13.37 ± 3.70	3.62
9	+ 0.68 ± 0.34	2.00	+ 17.38 ± 3.68	4.72
10	+ 0.85 ± 0.32	2.66	+ 2.15 ± 4.39	
Totals	+ 1.06 ± 0.16	6.62	+ 0.57 ± 1.69	
11	+ 1.64 ± 0.40	4.10	− 3.21 ± 4.26	

Variability in litter size is not as clear-cut as was the same constant in birth weight. There it will be recalled the tests, with the exception of two generations, were consistently less variable than the controls and the suggestion seemed plausible that if alcohol were acting as a selective agent the results secured were the ones to be expected.

In litter size Table IV shows that in the earlier generations (again omitting the second generation as being based on too few numbers) the tests are less variable than the controls, while in the later generations they are more variable. In the total for the ten generations, however, the difference between the two coefficients of variation is less than its probable error, so that it may be concluded that taken as a whole the experiment failed to show that a modification in variability of litter size had been induced.

Discussion

These results on litter size are in striking contrast to those secured by MacDowell (1921, 1922, 1922a).

MacDowell's data show a decrease in litter size of 10.5 per cent. in the tests of the first treated generation; a decrease of 10.3 per cent. for treated offspring of treated rats; a decrease of 11.2 per cent. for untreated offspring of treated rats; a decrease of 13.1 per cent. for untreated offspring of untreated parents and treated grandparents. These differences in all the above groupings are without statistical significance, but the number of cases is small and statistical significance (3.6 times the probable error) is only attained by taking all generations together.

MacDowell's results are consistent for all strains and all generations, but a valid difference is obtained only in the total of all strains and generations. This difference is just the reverse of our litter size data as shown in Tables III and IV and discussed above. Attempts to reconcile or explain this discrepancy seem to end in a blind alley. It remains, then, merely to point out several

differences between MacDowell's experiment and our own. Differences in the results obtained by the two investigations may or may not be due to these facts.

First. MacDowell's rats were treated during pregnancy so that the alcohol may have affected the young *in utero* either directly or indirectly. One plausible explanation of reduced litter size in the offspring of treated females would be a greater prenatal mortality in the litters of treated mothers than in the litters of the controls. That this is probably not the explanation, or only a minor element in the reduction of rats per litter, is evidenced by the fact that reduction in litter size continued for two generations after the treatment stopped. In this respect MacDowell's method of treatment departed widely from that reported here. Our animals were mated the day treatment stopped and no further alcohol was administered to such females. This limited our problem to a possible effect of the alcohol on the germ cells in the adult rat and excluded direct modification either of embryonic germ cells or the embryos themselves.

Second. MacDowell has nowhere published data on birth weight. As shown above, not only our own studies but those of other investigators as well strongly indicate that litter size and birth weight are negatively correlated. Reduced litter size may be compensated for by increased birth weight per animal, or *vice versa.* This is apparently the case in the third, sixth and tenth treated generations of our experiment where large litters are characterized by low birth weight per animal. In other generations this tendency is not evident.

It would be fortunate, indeed, if MacDowell has unpublished data on birth weight for his various series of animals and it could be ascertained if there is a difference between his tests and controls in the amount of *rat* produced during the same length of time and regardless of whether this total production of *rat* was in few or many individuals. Raw data for such a study by us are available and will be made.

Having pointed out this possibility it is now in order to state the case against the explanation lying here. And to us it seems that the case against the birth weight factor as explaining why MacDowell got reduced litters in his test animals and we got larger litters is stronger than the argument for it. The situation may be as simple as this. The primary fact to be reckoned with is the number of eggs ovulated and fertilized. If these be numerous the total maternal food supply will be distributed in smaller amounts to individuals than if only a few embryos are developing. Hence increased litter size would automatically reduce individual birth weight. Or is there some maternal regulatory mechanism which at the expense of the mother provides an increased food supply when the eggs released are numerous?

Third. It need not be dwelt upon, but our data are based upon exactly twice the number of litters (482) that MacDowell had (241). Furthermore, all our data are based on a single strain of inbred rats known at the Wistar Institute as the Tyler strain. The entire 482 litters produced by the control (244 litters) and treated (238 litters) are the offspring of a single sister-by-brother mating with which the experiment started.

MacDowell's 241 litters are not comparable to our 482 litters for one reason because his totals include four distinct strains (his A, B, C and LHKC strains); and these strains are still further subdivided into "treated from treated," "treated from untreated," "untreated from treated," "untreated from untreated from treated," etc. And it may be noted that some of his strains are outbred while others are inbred.

Nothing said above or hereafter is by way of criticism of either the methods or the findings of MacDowell's work. Our contrary results on litter size are so definite and striking as to merit a statement of any differences in procedure which might possibly have a bearing upon the matter.

MacDowell criticizes the controls of previous workers in similar experiments as merely "coming from the same dealer" or "being just normal and comparable," or "guaranteed not to be brothers and sisters." He goes on to say, "controls that may fully satisfy the demands of a psychologist or an anatomist may seem quite worthless to a geneticist; for the geneticist is more keenly aware of the great variety of heritable differences, the presence of which may vitiate the results, and *he demands the greatest possible similarity of germ plasm between tests and controls.* In the present work the brothers of the treated males and the sisters of the treated females were used as controls." (Italics ours.)

With these sentiments all geneticists will agree. However, the question may be raised whether MacDowell has fulfilled all the conditions of an adequate genetic control. He used brothers and sisters of the treated for controls, but if these are brothers and sisters within a heterozygous strain, and if Mendelian segregation be operating, then the use of brothers and sisters as controls is of but little more value than the controls condemned by MacDowell above. His line LHKC has a fourfold origin, starting from four unrelated rats in two pairs. Furthermore, the two pairs of ancestors were treated lightly with alcohol. The racial effect, if any, is believed to be slight, and in subsequent generations would be distributed equally among controls and treated.

The other strains used by MacDowell have a history but little different from his strain LHKC. The rats of the first series are the result of a cross made between stock secured from two different laboratories and believed to be unrelated. The second series of experiments was derived from three litters of Wistar Institute stock and from one mixed strain of rats from stock held over from the first series. Children and grandchildren of these hybrid strains were used as the original treated animals. Mendelian segregation must have been at its height when the alcoholic treatment began, and there

may be some question of the value of the controls in such a situation even though they be full brothers and sisters of the tests. This would not seem to fulfill the conditions of "the greatest possible similarity of germ plasm between tests and controls."

Another point may have considerable bearing on our diverse results. When MacDowell's strains are considered separately, the treated rats in strains A and C give smaller litter averages than the controls, but in strains B and LHKC the treated rats produce larger litters than the controls. And, again, dividing strain LHKC into groups on the basis of the generation of their parents, two of the three group averages favor the tests. It is only by taking all strains together that a ten per cent. reduction in the size of the test litters can be shown. If any one of the four strains or groupings in which the tests produce larger litters than the controls was greatly magnified in numbers the results might conceivably be in line with our own. At any rate the two experiments then would be essentially similar with the exception of the hybrid origin of all MacDowell's strains.

Stockard, and his coworkers, have published data on litter size and birth weight in guinea pigs. These data (from Stockard and Papanicolaou, 1918: tables 2 and 5 have been reduced to biometrical constants and published by MacDowell, 1922) show the same general results as those obtained by MacDowell. When reduced to ordinary biometrical constants and divided into seven groups—comparable to the groupings used by MacDowell—four of the treated groups have significantly smaller litters than the controls and in the other three there is an actual, though not significant decrease in litter size in the test animals.

Stockard's seven groups are all compared with one group of 88 control animals. As the seven groups of the treated represent several generations in time, the controls could not fulfill the required conditions of being brothers and sisters of the tests, and were obviously pro-

duced contemporaneously with only a small number of the alcoholics.

King (1924) shows clearly that the second litter tends to be the largest of the series cast by albino rats, while the first, third and fourth litters are but very little smaller, the average number of young per litter for the first four litters in King's data being 6.1, 6.7, 6.1 and 6.4, respectively. For all practical purposes then, litter size does not vary significantly for the first four litters.

Litter size in our data is computed on the early litters only. Enough breeding females were maintained in each generation so that one or two litters from each gave the required number of young. In a very few cases in the earlier generations some females produced a larger number of litters.

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A LETHAL MUTATION IN MAIZE AFFECTING THE SEED[1]

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A MULTITUDE of deleterious characters has been uncovered in the maize plant by the inbreeding now widely employed in improvement schemes. Among these variations, defective seeds are of frequent occurrence. Mangelsdorf (1926), who has given this subject careful study, has identified several genes which prevent normal development of the embryo and endosperm. He estimates that, on the average, about one plant in thirty within some common cultivated varieties of maize is heterozygous for a defective seed factor. The character has also been shown to arise *de novo* in pedigreed material (*cf.* Mangelsdorf, 1926). In the present paper we shall describe a further case of this sort. In understanding the present composition of maize and in predicting the probable stability of the sub-races now being developed by continued

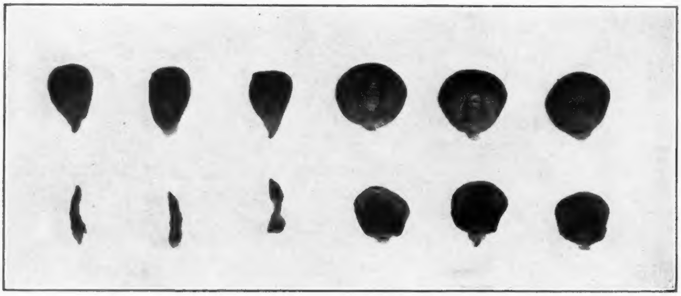

FIG. 1. Normal (above) and defective (below) seeds from ear R12–62. The grains at the left are shown in end view and those at the right in side view.

[1] Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 77. Published with the approval of the director of the station.

self-pollination, a knowledge of the frequency of such mutations is important. At present our information in this regard is very meager. It is in the hope that such isolated observations as are being made will indicate the direction in which a more comprehensive study could profitably be carried out that we record an additional case of mutation.

In 1924 a progeny designated R12, comprising sixty-four plants, was raised from a cross between an inbred strain of a variety of maize known as Burr's White Dent and a homozygous waxy stock. Self-pollinated ears were obtained on fifty-four individuals. Among the plants which were tested fifty-three produced normally filled ears; one individual, R12–62, proved to be segregating for a defective seed condition.

Defective seeds from the original ear are shown beside normal grains in Fig. 1. Examination of a larger range of material from the descendants of plant R12–62 reveals that embryo and endosperm develop to varying degrees of perfection in the defective seeds. In a few cases, the original ear being among them, sufficient endosperm reserves were formed to give the iodine reaction for starch. This has made it possible to recognize the four expected classes on certain ears segregating for both the defective and waxy characters. Usually, however, defective seeds are so ill-developed that classification for other endosperm conditions can not be made. The defective seeds can always be counted, however, although it is frequently necessary to first remove them from the cob with the point of a small knife. There is probably a small error involved in the counts, since all the ovules which for any reason have remained undeveloped are classed as defective. Defective seeds are quite incapable of germination. The character, therefore, is lethal.

A summary of the distributions in Tables II and III shows that among 10,977 seeds borne on selfed ears from segregating plants, 25.93 per cent. of the individuals were defective. It appears, therefore, that the defective char-

acter behaves as a simple recessive to the normal or non-defective condition. Following Mangelsdorf's (1926) scheme we designate the gene de_{15}. The observed ratio of defective seeds is actually 102 ± 31 seeds in excess of the proportion expected on the monohybrid basis. This apparently significant departure from a simple Mendelian ratio is probably a result of irregularities in pollen tube growth occasioned by the waxy factor. We shall advert to this situation again.

TABLE I

DISTRIBUTION OF SEEDS ON THE ORIGINAL EAR WITH RESPECT TO THE DEFECTIVE AND WAXY CHARACTERS.

	Number of seeds				
	Normal		Defective		
	Non-waxy	Waxy	Non-waxy	Waxy	Totals
Observed	213	14	23	52	302
Calculated (6·8 : 1 : 1 : 6·8)	207	18	18	58	301
Deviations	+6	−4	+5	−6	+1
	$X^2 = 3.07$		$P = 0.38$		

ORIGIN OF THE CHARACTER

The circumstances under which this character appeared suggest that it may have arisen as a mutation in the generation preceding that in which it was observed. The fact that but one plant among the fifty-four which were selfed segregated for defective seeds shows that neither of the parents of progeny R12 was heterozygous for the condition. The other possibility to be considered is that the individual which bore the original segregating ear, R12–62, was of different parentage from the other members of progeny R12; in spite of the usual precautions taken to avoid such an event, a stray pollen grain may have gained access to the silk and functioned in the production of the seed from which plant R12–62 was reared. The facts, however, do not favor this explanation. The plant in question proved to be segregating for waxy, like its sibs. Hence, if the defective gene was brought in by a stray pollen grain this latter must have arisen in an

individual carrying the waxy factor. The writer had not more than seventeen waxy plants in 1923 and these were grown about two hundred feet from the pistillate parent of R12 and separated from it by a block of sugary maize. The probability of accidental pollination from this source is very remote.

Plant number 62 showed no morphological peculiarities such that it attracted particular attention in progeny R12. Had it been different it would scarcely have escaped

TABLE II

RESULTS OF SELFING PLANTS OF THE COMPOSITION $WxDe_{15} \cdot wxde_{15}$ WHERE THE DEFECTIVE SEEDS CONTAINED SUFFICIENT ENDOSPERM TO ALLOW OF CLASSIFICATION FOR THE WAXY AND NON-WAXY CONDITIONS

Pedigree number	Year	Number of Seeds: Non-defective Non-waxy	Non-defective Waxy	Defective Non-waxy	Defective Waxy	Total	Per cent. waxy	Per cent. defective
R12–62 (Original ear)	1924	213	14	23	52	302	21.85	24.83
R74–31 ...	1925	222	35	39	26	322	18.94	22.98
–33 ...	1925	188	51	51	31	321	25.54	25.54
–90 ...	1925	147	17	22	25	211	19.91	22.27
Observed totals		770	117	135	134	1156	21.71	23.27
Calculated (3 · 02: 1: 1: 3 · 02)		741	126	126	163	1156	25.00	25.00
		$X^2 = 7.58$			$P = 0.057$			

notice, since R12 was being used in an experiment which required repeated and close attention to this group of plants. Furthermore, the stock was grown in an outside row on the breeding plot where a full view of the individual plants was readily obtained. The agreement between plant R12–62 and its sibs extended, moreover, to size and shape of ear, number of rows, form and color of seed. These facts point to the conclusion that the original plant segregating for the defective condition was of the same parentage as the other members of the progeny and that the defective gene arose by mutation in one of the parents.

LINKAGE RELATIONS

The defective seeds on ear R12–62 contained sufficient endosperm starch to permit of classification for the waxy and non-waxy characters by means of the iodine test. It will be recalled that waxy reserves stain reddish-brown with iodine in contrast to the blue reaction afforded by the common starch in non-waxy seeds. The frequency distribution with respect to both pairs of characters is given in Table I.

It is evident that the observed distribution in Table I does not conform to expectation on a simple dihybrid basis; the end terms in the ratio are disproportionately large and the middle terms are deficient. The frequencies of the four classes suggest linkage between the two pairs of characters. Applying Emerson's (1916) formula we obtain a gametic ratio of 6.8 : 1 : 1 : 6.8. This is equivalent to 12.8 per cent. crossing over. The goodness of fit of the ratio calculated on this basis to the observed distribution is fairly satisfactory, since P takes the value 0.38.

The defective gene, therefore, falls into one of the established linkage groups of maize comprising such well-known factors as I, i, (dominant white aleurone) C, c, (aleurone color) Sh, sh (shrunken endosperm) and Wx, wx, (waxy) whose linkage relations have been studied by Hutchison (1922). It has been shown more recently by Demerec (1926) that w_{11} (white seedlings) and d_3 (dwarf) also belong in this series. Hutchison (1922) found about 20 per cent. crossing over between sh and wx, and an effort has been made in the present investigation to determine the relative position of the de_{15} gene in the group by studying its behavior in crosses with these two factors.

In 1925 two progenies R74 and R75 were grown from the non-defective non-waxy and non-defective waxy seeds, respectively, on ear R12–62. The waxy progeny, R75, was crossed with a non-waxy stock, L11, and also with a line, R25a, homozygous for both waxy and shrunken. In R74, several plants were selfed and some further data immediately obtained on the behavior of de_{15} with reference to the waxy character (Tables II and

III). More extensive breeding facts were secured, however, in 1926 in the progenies grown from the outcrosses to waxy and shrunken lines. We shall consider first the additional results bearing on the behavior of the de_{15} with wx.

From the crosses between L11 and R75 four progenies were obtained which segregated for both the waxy and the defective characters. These lines are designated R145, R145a, R146 and R146a. The results obtained in selfing them are given in Table III. The frequency distributions from those ears in which it was possible to discriminate between waxy and non-waxy seeds in the defective class are also listed separately in Table II.

TABLE III

SUMMARY OF F_2 DATA FROM CROSSES OF THE TYPE Wx Wx De_{15} De_{15} x wx wx De_{15} de_{15}

Pedigree number	Year	Number of seeds: Non-defective: Non-waxy	Waxy	Defective	Total	Per cent. waxy in non-defective class	Per cent. defective
R 74 –14	1925	193	14	111	318	6.76	34.90
–93	1925	311	30	143	484	8.80	29.54
–98	1925	254	36	112	402	12.41	27.86
R145 –10	1926	264	20	95	379	7.04	24.93
R145a– 1	1926	291	28	110	429	8.78	25.64
R146 – 4	1926	350	30	125	505	7.89	24.75
– 5	1926	215	29	107	351	11.88	30.48
– 8	1926	312	25	102	439	7.42	23.23
–13	1926	309	43	106	458	12.18	23.14
R146a– 3	1926	269	33	129	431	10.93	29.93
– 5	1926	272	29	111	412	9.63	27.61
– 7	1926	265	27	89	381	9.24	23.36
Forward from Table I		770	117	269	1156	13.19	23.27
Observed totals		4075	461	1609	6145	10.16	26.18
Calculated (4 · 15 : 1 : 1 : 4 · 15)		4069	539	1536	6144	11.70	25.00
		$X^2 = 14.85$		$P = 0.0005$			

Estimation of the gametic ratio by Emerson's (1916) formulae from the observed zygotic distribution given in Table II leads to the proportion 3·02 : 1 : 1 : 3·02. Such a

ratio would result in a coupling series, with 24.9 per cent. crossing over. The goodness of fit between the zygotic distribution calculated on this basis and the observed values is poor, P being equal to 0.057. The amount of crossing over estimated by Yule's coefficient of association method (Collins, 1924) is 25.4 per cent. The use of this value, however, does not significantly improve the goodness of fit between the theoretical and observed distributions.

All the data relative to the linkage between de_{15} and wx have been assembled in Table III. The totals have been brought forward from Table II and the remaining items relate to the distributions on ears in which it was not possible to separate waxy and non-waxy individuals in the defective class. Estimation of the gametic ratio from these data by a minor modification of Emerson's method gives the proportion 4·15 : 1 : 1 : 4·15. This is equivalent to 19.4 per cent. crossing over. The goodness of fit between the observed ratio and that computed on the basis of the above gametic ratio is again very poor, since $P = 0.0005$. The coefficient of association method gives a crossover value in this case of 19.2 per cent.

The observed zygotic ratios in Tables II and III do not conform to the types which should result from the orderly distribution of linked factors, and hence satisfactory agreements with distributions calculated from any assumed gametic ratio are not to be expected. It will be noted from Table II that the observed proportion of waxy seeds is 21.71 per cent. or 3.29 ± 0.85 per cent. in defect of expectation on the monohybrid basis. This deviation is unquestionably significant and indicates that some factor is operating to disturb the waxy ratio. Irregular ratios have frequently been observed in maize strains segregating for the waxy gene. The explanation appears to lie in a differential rate of development of waxy and non-waxy pollen tubes (Brink, 1925). Since the defective gene is linked with waxy, we should expect some modification in the proportion of defective zygotes in these stocks also.

The F_2 results of the crosses between the lethal-carrying stock and the shrunken variety (R25a x R75) are given in Table IV. The linked genes in this case are present in the F_1 in the repulsion phase. Inspection of the observed zygotic ratios shows at once that the sh and de_{15} genes do not behave independently. The gametic ratio calculated by Emerson's method is 1:5·05:5·05:1, which is equivalent to 16.5 per cent. crossing over. The coefficient of association formula gives the same result.

TABLE IV

F_2 RESULTS FROM THE CROSS Sh Sh De_{15} de_{15} x sh sh De_{15} De_{15}

Pedigree number	Number of Seeds: Non-defective: Non-shrunken	Number of Seeds: Non-defective: Shrunken	Number of Seeds: Defective	Number of Seeds: Total	Per cent. shrunken in non-defective class	Per cent. defective
R144 – 1	229	143	123	495	38.44	24.85
–10	219	89	102	410	28.90	24.88
–20	241	125	124	490	34.15	25.31
–25	190	95	95	380	33.33	25.00
–29	258	118	158	534	31.38	29.59
– ?	262	96	117	475	26.81	24.63
R144a– 4	225	119	127	471	34.59	26.96
– 8	267	135	117	519	33.58	22.54
–11	297	110	144	551	27.03	26.13
R144b– 1	261	116	130	507	30.77	25.64
Observed totals	2449	1146	1237	4832	31.88	25.60
Calculated (1: 5 · 05: 5 · 05: 1)	2449	1175	1208	4832	33.33	25.00
	$X^2 = 1.41$		$P = 0.51$			

Since the value of P is 0.51 the fit between the observed and calculated zygotic distributions is close.

THE ORDER OF THE GENES

The best estimate of the amount of crossing over between wx and de_{15} with the data available is 19.4 per cent.; sh and de_{15} show about 16.5 per cent. crossing over. Hutchison (1922) found approximately 20 per cent. crossing over between sh and wx. It must be borne in mind in using these figures that they are based upon results from genetically different stocks which may not

give closely comparable crossover values for the regions in question. The critical test in determining the order of the genes, back-crossing the triple heterozygote with the triple recessive, is not possible with this material, since de_{15} is lethal in the homozygous condition. The evidence indicates, however, that the de_{15} gene lies roughly midway between the sh and wx loci. This arrangement calls for about one and one half times as much crossing over between sh and wx as Hutchison observed. Until further genes occupying loci in this region of the chromosome are available for testing some doubt must remain

TABLE V

F_2 DISTRIBUTIONS ON EARS FROM THE CROSS BETWEEN THE De_{15} de_{15} STOCK AND COULTER'S RACE APPARENTLY NOT SEGREGATING FOR DEFECTIVE SEEDS

Pedigree number	Non-defective: Non-waxy	Non-defective: Waxy	Defective	Total	Per cent. waxy in non-defective class	Per cent. defective
R143–14	272	109	26	407	26.68	6.39
–19	309	75	7	391	19.53	1.79
–24	282	106	19	407	27.32	4.67
Totals	863	290	52	1205	25.15	4.31

as to the relative position of these three factors, but the probable order is sh – de_{15} – wx.

RELATION OF DE_{15} TO COULTER'S ZYGOTIC LETHAL

To account for irregular aleurone color ratios encountered in certain of his stocks, Coulter (1925) has postulated a zygotic lethal gene (l_1) linked with the c factor. It was estimated that c and l_1 are about eighteen units apart. Since c is a member of the same linkage group as sh and wx with which de_{15} is linked it is of interest to determine the relation between the latter factor and l_1. Dr. Coulter kindly furnished us with some of his material and this has been tested with our de_{15} stock.

In 1925 the progeny carrying the l_1 factor did not

thrive at Madison and was, moreover, badly damaged at a critical time by a storm. No self-pollinations were successful, but three ears were secured from crosses with two plants in progeny R75 used as pollen parents. In view of their breeding it was expected that the plants in R75 were mostly heterozygous for de_{15}; this composition was proved by self-pollination, however, in the case of but one of the individuals used in the cross. The three hybrid ears obtained bore, respectively, 30.20 per cent., 32.80 per cent. and 25.84 per cent. defective seeds. This result might be taken to indicate that Coulter's

TABLE VI

F_2 DISTRIBUTIONS ON EARS FROM THE CROSS BETWEEN THE De_{15} de_{15} STOCK AND COULTER'S RACE WHICH ARE SEGREGATING FOR DEFECTIVE SEEDS

Pedigree number	Non-defective: Non-waxy	Non-defective: Waxy	Defective	Total	Per cent. waxy in non-defective class	Per cent. defective
R143 – 9	138	32	157	327	18.82	48.01
–24	186	16	127	329	7.92	38.84
R143a– 7	135	35	208	378	20.59	55.03
– 9	84	16	190	290	16.00	65.52
–17	161	22	189	372	12.02	50.81
Totals	704	121	871	1696	14.67	51.36

lethal is identical with our own and that the observed proportions of defective seeds are approximations to a monohybrid ratio. The F_2 results, however, do not indicate that such an explanation is sufficient.

In 1926 two progenies, R143 and R143a, respectively, were grown from the normal seeds on two of the above hybrid ears. Eight plants were self-pollinated. The ears produced fall into two classes with respect to proportion of defective seeds formed. The first group, as shown in Table V, bore less than five per cent. of defective seeds which may have been the result of incomplete pollination or other such circumstances. The five ears

listed in Table VI, on the other hand, produced 51.36 per cent. aborted seeds on the average. This result can not be explained on the hypothesis that only one lethal factor is operating. It is possible that Coulter's stock contains two lethals, one of which is identical with de_{15} but the available evidence is inadequate to establish such a relationship. Since Coulter (1925) was unable to identify the defective seeds which were presumed to occur in his stock, the frequency of this class is not given in his paper. In the present connection these data would be of critical value.

SUMMARY

A lethal mutation (de_{15}) in maize affecting seed development is described. The gene occupies a locus in chromosome I and shows 19.4 per cent. crossing over with wx and 16.5 per cent. crossing over with sh. It is provisionally concluded that the order of the factors is sh – de_{15} – wx.

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POLYANDRY IN THE GROUSE LOCUST, *PARATETTIX TEXANUS* HANCOCK, WITH NOTES ON INHERITANCE OF ACQUIRED CHARACTERS AND TELEGONY[1]

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AN idea persists usually for one of two reasons: (1) because it is true and workable, or (2) although apocryphal, it is plausible to the average person who may possibly make useful application of it in some indirect way. Thus the idea of the inheritance of acquired characters[2] holds out in the face of some critically experimental evidence against, many discredited efforts to establish, and *no independently confirmed results supporting its validity.*

It is reported that a ranchman persistently painted his barns, fences and even the lower trunks of the trees in the barnyard, red. He did this with the expectation that the intensely red environment would influence the pregnant cows in such a way that their calves would have a greater tendency towards redness, the color idealized for his herd. It was confidently claimed that the development of the splendid herd of red quality cattle had been due to this measure.

No one should be under any illusion as to the actual factors which contributed to the consummation of this ideal product. To the man who wanted red cattle so ardently, the red environment had been as the constant, fervent and ever reminding prayer towards the fulfillment of which he had bent, largely unconsciously, all his resources in selecting for breeders, trading with others, buying and

[1] Paper No. 90, Zoology Department, Kansas State Agricultural College and Experiment Station.

[2] Inheritance of acquired characters is here defined as the alleged continuance in the posterity of somatic modifications induced directly by the environment, or indirectly through the experimental alteration of the germ plasm, and without the further application of the modifying agency.

selling. In such ways the, as yet, entirely unsupported proposition of the inheritance of acquired characters has probably been, and perhaps, still remains an invaluable expedient, operating as a subconscious incentive to effective selection in the improvement of plants and animals under domestication, and in the still more complicated affairs of human education and progress.

The idea of telegony ("The alleged influence of a previous sire on the progeny of a subsequent one from the same mother") also endures against experimental disproval and failure of substantiation in any way. A good review of the subject of telegony, with a sense of the futility of the proposition, is contained in an article by Dr. E'tienne Rabaud, in *Biologica,* May 15, 1914 (Translated in *The Journal of Heredity,* 5: 389–399, 1914).

In the foot-note to the translation of this paper, the editor of *The Journal of Heredity* states that "most cattle, horse and swine breeders, as a rule, still adhere to the idea (telegony), and several sheep breeders' associations even refuse to register lambs whose mothers were ever 'impregnated' by mating with a common ram."

Such a restriction imposed by dog and sheep breeders' associations is indubitably just another variety of credulity. Nevertheless, men who are careless, or thriftless to the extent that they allow valuable bitches or ewes to mate with common dogs or rams, respectively, are not usually sufficiently responsible to be trusted with the application of the *essential* rules of an effective association. It is also likely that persons composing the lower fringes of the responsible body of breeders are encouraged by the, to them, reasonableness of the idea of telegony to make better efforts than they otherwise would. It is further conceivable that, among average men of conviction, the one who believes in telegony might be a more trustworthy breeder of high-grade stock than the one who knows better.

Undoubtedly such ogreisms as the inheritance of acquired characters and maternal impressions, telegony

and other plausible superstitions, operating as expedients, have had, and still have disciplinary values of much greater force and considerably wider range than are commonly accorded them by biologists.

Polyandry in *Paratettix texanus*

Hancock ('02) states that polyandry was frequently observed among the *Tettigidae,* mostly in the genus *Acridium* (The *Tettix* of Hancock), and other grouse locusts that spent short and frequent intervals in copulation. Those that required longer periods at a time for the act of mating, such as the genus *Tettigidea,* were more likely to be restricted to the one, or fewer males for each female.

Polyandry has been noted among *Apotettix eurycephalus* and *Paratettix texanus* during the fifteen and eighteen years, respectively, of their use in experimental breeding. In 1923–24, Miss Caroline M. Perkins, Mr. E. H. Ingersoll and the author carried on a series of special experiments with *P. texanus* which would indicate the possibilities of this species in polyandrous matings. The mere curiosity to determine how many males might take part in the parentage of the progeny of given females was the main motive. Anyway the regular program of study of gametogenesis in both the males and females would not be interrupted. If the sexual products of any male or males were to affect the color patterns or other observable features of the progeny of another male in a polyandrous mating the fact would be noted. However, the negative results of several experimenters using other materials (Rabaud '14), and the consequences of extensive previous breeding of the grouse locusts did not render such an outcome probable.

Eight females of *P. texanus* were placed separately in mating jars, and each given opportunity to mate with several males of *different dominant contrasting color patterns, and the normal recessive,* of such kinds and in such ways that there would be no doubt of the identity

of the paternity of the offspring. They were exposed to males as follows: one to five different males; one to four; four, each to seven; one to nine, and one to eight males.

To five of these females the males were mated successively; that is, only one male was placed with a female at a time and, as soon as copulation occurred, he was removed and another introduced. No male was allowed to mate with a given female more than once. These five females with which four (exposed to five), three (exposed to four), seven, eight (exposed to nine), and seven males copulated successively, and only once each, respectively, gave offspring as follows: Female number 1, (+/J), 44 showing parentage of three males; female number 2, (+/Sm), 70 indicating parentage of three; female number 3, (+/C), 260 demonstrating participation by five males; female number 4, (+/+), 139 revealing five males as parents, and female number 5, (+/+), 75 offspring with four males taking part (Table I, Females number 1 to number 5).

Seven, seven and eight males, respectively, were placed all at the same time with the other three of the separated females. They were kept under observation and notes made of the time each male spent in copulation. When the observer had to be absent, the females were segregated from the males. All seven males with female number 6 mated with her during a 14-day period; two of them once on one day each; one once each, on two days; one once each, on four days, and two on seven days each, respectively.[3] The seventh is recorded as only trying (five times) to effect copulation and apparently not succeeding; but the observer's attention must have been diverted, at least once, for the records of the 102 offspring show that he, as well as the other six males, actually took part in the parentage. The next female, number 7, mated to six males, which were all with her over most of the period of 22 days, gave 186 offspring having the charac-

[3] Male BHm/S copulated with female number 6 four times on the eighth day. The small figures throughout indicate the number of times copulation occurred on that day.

ters of four of the males. The last female number 8, exposed to eight males, seven of them copulating with her, gave 169 offspring from four of the males. (Table I, Females number 6 to number 8).

Discussion

Females numbers 3, (+/C), 4, (+/+), and 5, (+/+), gave overwhelming majorities of offspring from the males that mated with them last, +/+, on the ninth day, D/P, on the thirteenth day, and Cof/S, on the sixteenth day, respectively. Female number 7, (+/+), also gave majorities from those males, Hm/P and +/+, that mated with her on the twentieth and twenty-first days. On the other hand, female number 8, (+/+), gave more offspring from each of males +/+ and F/J, that mated with her on the twentieth and twenty-first days, respectively, than from male Cof/Sm that mated twice on the twenty-second day. The male E/I, that mated nine times on five days, and male D/P, that copulated five times on four days, did not become parents at all.

Explanations of Symbols used in the Table

The color patterns represented by the following factor symbols to which reference is made in the table are illustrated in the *Journal of Genetics*, Vol. III, No. 1, 1917, Plate II: +/+, "normal recessive," = (AA, symbol changed); +/B = BB; +/C = CC; +/Cof = (QQ, symbol changed); +/D = DD; +/E = EE; +/F = FF; +/H = HH; +/I = II; +/J = JJ; +/P = PP; +/S = SS; +/Sm = ($I\widehat{S}I\widehat{S}$, symbol changed); C/J = CJ; D/J = DJ; C/S = CS; C/I = CI; B/C = BC; Plate I (ob. cit.); C/H = CH; I/Sm = $I\widehat{IS}$. The illustrations of the following have not yet been published: K = narrow white stripe (resembles K of *A. eurycephalus*, Kansas Tech. Bull. 17, Plate I); Hm = less orange, more grey than H; N, grey-brown-all-over; N_2, red-all-over.

In the tables, the figures following the males, 7th, 6th, 10th, etc., females numbers 1 to 5, indicate the day of the period on which copulation occurred. Females numbers 6 to 8 had all the males with them during most of the respective periods. For example, male E/1 mated with female 8 (+/+), once each on the 10th, 11th, and 16th days, three times each on the 15th and 17th days; indicated by the "3" following the 15 and 17, respectively.

Under the heading of offspring, the number of males is on the left, and females on the right of the hyphen (-). For example, male I/Sm produced, with female number 3, +/I, 6 males, 7 females; +/Sm, 4 males, 3 females; C/I, 4 males, 4 females; C/Sm, 5 males, 1 female.

TABLE I

Polyandrous Matings in *Paratettix texanus*

Females (1) to (5): Males copulated only once each.
Females (6) to (8): Males with these during most of periods indicated

Males	Days of 8-day Period on which Copulation occurred	(1) Female	Offspring		Males	Days of 16-day Period on which Copulation occurred	(5) Female	Offspring	
Cof/K	2nd		+/Cof	2– 2	E/I	5th		+/N	0– 2
			+/K	3– 0					
C/Hm	3rd		Cof/J	4– 1	N/N_2	6th		$+/N_2$	1– 0
			J/K	1– 2					
D/Sm	6th		+/C	0– 4	B/H	7th		+/P	1– 0
		+/J	+/Hm	2– 1			+/+		
N_2/S	7th		C/J	2– 2	K/P	8th		+/D	1– 2
			Hm/J	2– 2					
B/H	0		+/Sm	3– 5	D/J	9th		+/Cof	22–16
			D/J	2– 2					
			J/Sm	1– 1	+/+	10th		+/S	17–13
			Total	44	Cof/S	16th		Total	75
	7-Day Period	(2) Female				14-Day Period	(6) Female		
C/Hm	1st		+/C	6– 4	B/K	1^3, 2, 3^2, 8, 9, 10^2, 11		+/B	5– 4
			+/Hm	9– 2				+/K	3– 1
Cof/K	3rd		Hm/Sm	5– 1	I/Sm	1		+/I	5– 3
		+/Sm	+/Cof	1– 2			+/+	+/Sm	5– 4
N_2/S	7th		+/K	1– 2	BHm/S	1^2, 3, 7^2, 8^4, 9^2, 10, 11		+/BHm	1– 0
			Cof/Sm	2– 4				+/S	2– 0
B/H	0		K/Sm	1– 0	N/N_2	2, 7, 9, 12		+/N	6– 4
			$+/N_2$	5– 5				$+/N_2$	1– 7
			+/S	4– 3	H/Hm	3, 8		+/H	5– 6
			N_2/Sm	2– 2				+/Hm	12– 2
			S/Sm	6– 3	Cof/J	11		+/Cof	4– 2
								+/J	7– 4
			Total	70	+/+	Five attempts only, observed		+/+	1– 8
								Total	102

TABLE I—*Concluded*

Polyandrous Matings in *Paratettix texanus*

Females (1) to (5): Males copulated only once each.

Females (6) to (8): Males with these during most of periods indicated

	9-Day Period	(3) Female				22-Day Period	(7) Female		
I/Sm	1st		+/I	6– 7	E/I	3		+/Hm	22–21
			+/Sm	4– 3					
B/K	3rd		C/I	4– 4	Hm/P	9, 13, 14, 17, 18, 19, 21		+/P	25–28
BHm/S	4th		C/Sm	5– 1	B/N_2	9, 10, 11[2]		+/S	1– 1
		+/C	+/B	3– 0			+/+		
N/N_2	4th		+/K	2– 3	Cof/S	9[2], 12, 15[3], 18, 19		+/+	40–46
			B/C	3– 0					
D/J	5th		C/K	0– 1	+/+	10, 11, 14, 15, 20		+/J	1– 1
			$+/N_2$	1– 0					
H/Hm	6th		C/N_2	1– 1	J/Sm	11, 18[3], 20		Total	186
			+/H	0– 2					
+/+	9th		+/Hm	0– 1	H/K	0			
			C/H	3– 1					
			C/Hm	2– 0					
			+/+	53–57					
			+/C	41–51					
			Total	260					

	13-Day Period	(4) Female				22-Day Period	(8) Female		
B/K	3rd		+/B	1– 2	Cof/Sm	9, 14, 16, 22[2]		+/Cof	6– 6
J/N_2	7th		+/K	3– 2	E/I	10, 11, 15[3], 16, 17[3]		+/Sm	8–11
E/I	8th		+/Cof	0– 2	+/+	11, 14, 15, 20		+/+	17–21
H/Hm	8th		+/+	9–11	D/P	12, 16[2], 21, 22		+/F	19– 9
Cof/N	8th		+/S	2– 0	F/J	13, 17, 21		+/J	21–19
+/+	9th		+/D	31–34	B/K	14[2], 15, 20, 21, 22		+/B	9– 7
C/S	11th		+/P	23–19	H/Hm	22		+/K	7– 9
D/P	13th				Cof/N	0			
Cof/S	0		Total	139				Total	169

Several males were mated each to more than one female: The same male, E/I, was with females numbers 4, 5, and 7; Cof/K, 1, 2; C/Hm, 1, 2; N_2/S, 1, 2; B/H, 1, 2; I/Sm, 3, 6; B/K, 3, 4, 6; BHm/S, 3, 6; N/N_2, 3, 5, 6; D/J, 3, 5; H/Hm, 3, 4, 6; D/P, 4, 8. The male E/I proved sterile with three females. B/H did not copulate with females numbers 1 and 2, copulated with number 5, but gave no offspring. BHm/S gave no offspring from number 3, and only three from thirteen matings with female number 6. D/J was sterile with number 3 and gave three offspring with female 5. D/P gave a numerous progeny with female 4, and none with female number 8. The other males, on the whole, appeared to do about as well with the one as the other female.

Conclusions

1. The last male or males copulating with a female tended to become the parents of the predominant proportion of her offspring.

2. All seven males which were placed with one female, 6, concurrently, during a fourteen-day period, shared, respectively, in the parentage of her 102 offspring.

3. The sexual products of none of the males appeared to have any effect upon the observable characteristics of the progeny of any other males of polyandrous matings.

A SIRE'S BREEDING INDEX WITH SPECIAL REFERENCE TO MILK PRODUCTION

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CATTLE breeder and geneticist alike have recognized the importance of suitable methods for measuring a sire's breeding ability, particularly for those economic characters such as milk, percentage of fat and total fat which come to expression in the female alone. Although several have been proposed none are wholly satisfactory. Thus the progeny test, *i.e.,* the production of the daughters themselves, is an expression of the joint effort of sire and dams, but does not distinguish between the contribution of each. The use of the difference between dams' and daughters' mature average production as a measure of the sire's breeding ability encounters the serious objection that a sire mated to exceptionally good cows is handicapped compared to a sire of equal ability mated to mediocre or poor cows.

These objections are avoided by the method in use at Mount Hope Farm, which employs as an index of the sire's breeding ability the dams' average production plus or minus the weighted difference between this average and the daughters' average according as the latter is greater or less than the former.

Experimentally the formula for this index for milk quantity and fat percentage (to be stated in a moment) rests upon the observations of Gowen, Castle, Cole and others on crosses between breeds, one of which is more productive than the other. These workers report that a given level of milk yield or fat percentage is not completely dominant over lower levels, since the offspring are intermediate between parental breed levels. However, neither milk quantity or fat percentage are exactly half

way between the parental breed levels. Milk quantity is somewhat nearer the level of the higher parent breed, fat percentage somewhat nearer the level of the lower parent breed. These qualitative statements may be converted into a quantitative form, which states that the quantity of milk produced by a cross between breeds is seven tenths (approximately) of the difference between the parental levels above the level of the lower parent. Fat percentage is four tenths (approximately) of the difference above the lower parent. These relationships make it possible to determine either sire's, dam's or progeny's level when the other two are known.

In ordinary breeding operations it is not possible to obtain a sire's production directly as can be done for his mates and their daughters. But by extending the observations on cross matings to ordinary matings a formula may be derived for a sire's breeding index for each character. The records are first converted to a common age basis (mature form). The daughters' and their dams' records are then averaged separately. The sire's breeding index for milk quantity is the dam's average production plus 1.4 times the difference between the dams' and daughters' average when the latter is the greater. When it is less, then 3.3 the difference is subtracted from the dams' average to get the sire's breeding index. For fat percentage the multipliers are 2.5 and 1.7, respectively. The index for total fat is the product of the milk quantity index multiplied by the fat percentage index.

The arithmetic for calculating a sire's breeding index for milk quantity may be illustrated as follows:

First instance: Daughters' average record (in pounds) higher than that of their dams.

12000 (daughters) — 10000 (dams) $= 2000 \times 1.4 = 2800$.
$10000 + 2800 = 12800$, sire's breeding index.

Second instance: Daughters' average record lower than that of their dams.

12000 (dams) — 10000 (daughters) = 2000 × 3.3 = 6600. 12000 — 6600 = 5400, sire's breeding index.

The arithmetic for fat percentage is equally simple and need not be illustrated.

The multiplying factors, which are the reciprocals of the several tenths of the cross breeding experiments, are to a certain extent provisional. However the trials of a series of multipliers which have been made indicate that the values selected are satisfactory.

This breeding index is of course subject to the effects of random sampling. Its usefulness may also be impaired by inequalities in the environment, especially when dams' and daughters' records are made under radically different conditions. These difficulties, however, are not peculiar to this breeding index but are common to those mentioned above.

Application of the formula has been made to a series of 68 Jersey sires selected from Maine Experiment Station Bulletin No. 281 and to the series of 58 sires of unknown breed which is listed in U. S. Department of Agriculture circular 368. They are also being used in a

TABLE I

MILK QUANTITY

Name	Pairs	Dams' Average	Daughters' Average	Difference	Weighted Difference	Sire's Index
Itchen King	7	15471	18680	+ 3209	+ 4493	19964
Langwater Africander	16	13554	14648	+ 1094	+ 1532	15086
Langwater Knight	6	16859	16878	+ 19	+ 27	16886
Langwater Traveller	14	10856	13776	+ 2920	+ 4088	14944
Langwater Warrior	20	15390	14888	− 502	− 1657	13733
Masher's Elsie's May King	7	13424	13858	+ 434	+ 608	14032
Maxim of Linda Vista	11	11578	14389	+ 2811	+ 3935	15513
Noble Regent of Allenwood	5	15719	16652	+ 933	+ 1306	17025
Sir Snowdoun	18	9081	10304	+ 1223	+ 1712	10793

TABLE II

FAT PERCENTAGE

Name	Dams' Average	Daughters' Average	Difference	Weighted Difference	Sire's Index	Sire's Total Fat Index
Itchen King	4.73	4.64	–.09	–.15	4.58	914
Langwater Africander	4.84	5.01	+.17	+.43	5.27	795
Langwater Knight	5.08	4.78	–.30	–.51	4.57	722
Langwater Traveller	5.06	5.18	+.12	+.30	5.36	801
Langwater Warrior	4.85	4.76	–.09	–.15	4.70	647
Masher's Elsie's May King	5.35	5.51	+.16	+.40	5.75	807
Maxim of Linda Vista	4.89	5.21	+.32	+.80	5.69	883
Noble Regent of Allenwood	5.53	4.97	–.56	–.95	4.58	780
Sir Snowdoun	5.08	4.97	–.11	–.18	4.90	530

study of Guernsey sires, several of which, for illustrative purposes, are listed in Tables I and II. Though for the most part these Guernsey bulls are superior animals due to the method of selection, a glance at the tables shows how well the breeding index takes into account both the quality of the cows with which a bull is mated and his reaction with them. Thus Langwater Knight was mated to a very superior set of cows. Though he was not able to raise their records appreciably, he showed his quality by siring daughters whose records equaled their dams. Compare his performance with that of Langwater Traveller whose daughters' record exceeds that of their dams by the large amount of 2,900 pounds. But when the mates of Langwater Knight are compared with those of Langwater Traveller the latter are found to have relatively low records. The proposed breeding index reflects the behavior of these two bulls with relation to the average

ability of their mates, the former having an index of 16886, the latter one of 14944.

Or compare Itchen King, Noble Regent of Allenwood and Langwater Warrior, each mated to cows whose average records are approximately equal. (See Table I.) The breeding indices of 19964, 17026 and 13733 reflect closely the reactions of these three bulls as shown by their daughters' records. Now though Langwater Warrior has the lowest index of the three, and though his daughters did not equal their dams' records by 502 pounds, his breeding index is only 299 points lower than that of Masher's Elsie's May King whose daughters' average record was 434 pounds above that of their dams, but was 1,030 pounds less than the average record of Langwater Warrior's daughters. The progeny test alone would make Langwater Warrior superior to Masher's Elsie's May King. The test by difference between dams' and daughters' production makes Masher's Elsie's May King superior. The breeding index shows that they are about equal.

When Sir Snowdoun's breeding behavior, as shown in Table I, is considered, it is quite evident that though he performed a real service in the herd in which he was mated, yet he is far from being the equal of Langwater Warrior, even though the latter lowered his daughters' record below that of their dams while the former raised his.

In this preliminary paper, an extended consideration of sires is impossible, but the studies in progress give good reason for believing that the proposed index represents an advance over those previously in use.

Further discussion is postponed for a more extensive paper. It remains only to add that the principles involved in this index may prove useful in other instances such as weight and size of animals or their organs, when the characters concerned are inherited on a multiple factor basis as milk production seems to be, and where more exact Mendelian analysis is impracticable.

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THE PIGMENTATION OF HUMAN HEAD-HAIR

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AFTER a hair-shaft has emerged from its follicle, and projects above the level of the epidermis, it may be termed a mature hair. Studies of such mature mammal hairs have already shown that the cuticular scales, medullas, pigment granules, and but rarely the cortical cells, bear well-defined relationships to the diameters of the hair-shafts in which they occur, or to the species of mammal bearing the hair. The studies alluded to have shown, namely:

1. That among *infra-hominid* mammals[1] the form of the cuticular scales is correlated directly with the diameter of the hair-shaft, as is also the form of the medulla;[2] but that the pigmentation characters are related to the species.[3]

2. That in man, the cuticular scale form is constant, but the scale size (*i.e.*, the proximo-distal diameter of the free surface of the scale) varies directly with the diameter of the hair-shaft; and the medulla forms (variations of a single type) vary with the diameters of the hair-shafts in a similar way.[4]

Since scales and medullas vary with the diameter of the hair-shaft, any hair may show different types of these structures (or variations of these types) in different regions of the shaft. And it was found that the pigmentation characters of *infra-hominid* hairs likewise

[1] A term employed to designate the mammals below the *Hominidae*.

[2] Hausman, L. A., "Further Studies of the Relationships of the Structural Characters of Mammalian Hair," AMERICAN NATURALIST, Vol. 58, November–December, 1924, p. 544.

[3] *Ibid.*, "Hair Coloration in Animals," *The Scientific Monthly*, March, 1921, p. 215.

[4] *Ibid.*, "A Comparative Racial Study of the Structural Elements of Human Head-hair," AMERICAN NATURALIST, Vol. 59, November–December, 1925, p. 529, and "The Relationship of the Microscopic Structural Characters of Human Head-hair," *Am. Jour. Phys. Anthropol.*, Vol. 8, April–June, 1925, p. 173.

showed differences existing from the proximal to the distal ends of the shafts. However, in this case, these pigmental differences were not correlated with differences in the diameters of the hair-shafts, as was true of the differences of scalation and medullation.

Scale and medulla form, then, in all mammals, is correlated with hair-shaft diameter. Pigmentation characters, in the *infra-hominid* mammals, are related to species (though within the species a secondary relation obtains with the regions of the shaft).

With what is the pigmentation of human hair correlated? This paper sets forth the results of a study of this matter through the examination of samples of human head-hair, gathered from many sources[5] and representing all the existing races of mankind.

Color, in *infra-hominid* hairs, is produced: (1) by a diffuse yellowish, or reddish-yellow, acid-soluble melanoprotein, which uniformly stains the keratized protoplasm of the cortical cells, and sometimes the cells of the medullary column; (2) by granules or large masses, of varying shades of brown, of an acid-soluble melanin substance, occurring in or among the cells of the cortex, or of the medulla, or of both; (3) rarely by a deposit, from skin glands, of a yellowish homogeneous pigment on the surface of the hair-shaft and (4) by a combination of all of these, most often of the first two. Hair colors are modified by the manner in which the light is reflected and refracted by the keratized and transparent protoplasm of the medullary and cortical cells and the cuticular scales.

Human hairs owe their colors to similar diffuse cortical and medullary stains and cortical pigment granules. Granules were found but seldom in the medullary column, and were never encountered here in such large dense masses and were frequently seen in the hair-shafts of *infra-hominid* species of mammals (Fig. 1).

[5] The writer heartily thanks the following persons for their kindness in sending him samples of head-hair: First and foremost, Dr. Aleš Hrdlička, then Dr. Chi Ping, Dr. Arnold Lundie, Professor E. W. Stafford, The Metropolitan Museum of Art, Dr. T. C. Nelson, Dr. H. D. Reed, Dr. A. L. Kroeber and Dr. F. G. Speck.

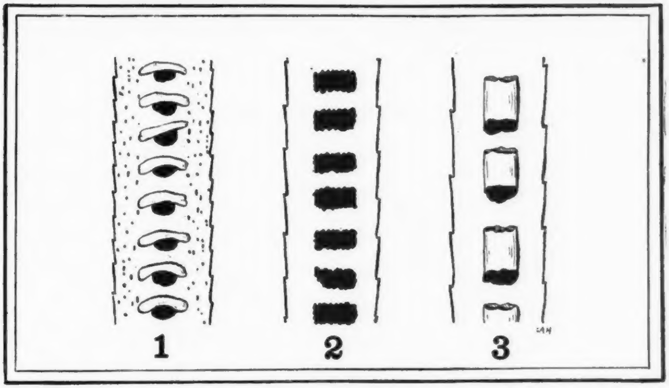

FIG. 1. Pigment masses in the medullary columns of: 1, the tip of the fur hair of the Blarina (*Blarina brevicauda*); 2, the median region of the hair of the Sewellel (*Aplodontia californica*); and 3, the base of the fur hair of the Muskrat (*Fiber zibethicus*).

The diffuse yellowish cortical stain of human hair, when present in small quantities, gives to the hair a light yellowish or creamy hue. In increasing amount it renders the hair increasingly "red" or coppery, and these colors are further deepened by the addition of cortical pigment granules, producing the familiar Titian and chestnut shades. Brown hairs usually contained, besides the cortical granules, some diffuse cortical stain as well.

The melanin pigment of human hairs in the form of discrete granules was found usually to be restricted to the cortex of the hair-shaft; sometimes it was encountered within the medullary column; and in Chinese and Japanese hairs (though here but seldom) it was seen occurring in the form of unusually large ovate granules scattered about sparsely in the transparent keratin of the cuticular scales (Fig. 2). Occasionally a hair-shaft was found in which the pigment granules were aligned along one side of the cortex only, leaving the other quite unpigmented and clear. It is believed that such a condition is correlated only with some abnormality of the papilla of the hair.

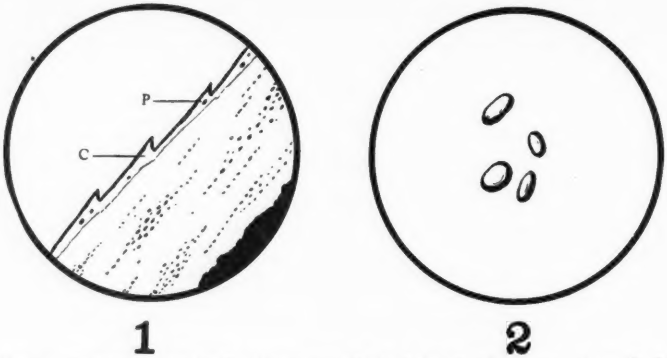

FIG. 2. Pigment granules in the cuticle of the hair of a Japanese. 1, optical section through a portion of the hair-shaft midway from the base to the tip. C, cuticle; P, pigment granules. A portion of the medulla is shown at the right. 2, the granules more highly magnified.

The pigment granules of human hair are roughly ovate or elliptical, and many variations of these forms are found (Fig. 3, 1). However they do not vary widely in shape, and no such irregular granules as one meets with in the cortices of *infra-hominid* mammal hairs were encountered in the human material (Fig. 3, 2–3).

In size, the human granules measured from 0.20 microns to 1.25 microns along their major diameters. Larger masses than these were found to be the result of the coalescence of two or more "elemental" granules. The largest granules occurred in hairs from members of the Bantu tribes of the African Negroid group, and the smallest in the light yellow hairs of Norwegians.[6]

[6] The ethnographic classification used in this paper is as follows:

- I. European Race
  - 1. South Mediterranean Branch
  - 2. North Mediterranean Branch
- II. African Race
  - 1. Negrillo Branch
  - 2. Negro Branch
  - 3. Negroid Branch
- III. Asiatic Race
  - 1. Sinitic Branch
  - 2. Sibiric Branch
- IV. American Race
  - 1. Northern Branch
  - 2. Central Branch
  - 3. Southern Branch
- V. Oceanic Race
  - 1. Negritic Branch
  - 2. Malayic Branch
  - 3. Australic Branch

The pigment granules are usually grouped into definite patterns in the cortex of the hair-shaft, and these patterns bear a definable relationship to the colors of the hair (Fig. 4). In the light yellowish hairs, that is the Cartridge Buff (19″ YO–Y f),[7] these patterns are linear; but as the hair increases in color-depth the patterns become elliptical and then ovate (Fig. 5). Many granules not connected with the pattern groups are found scattered about among the cortical cells, and often the linear patterns of granules are found in the darker hair-shafts where the elliptical and ovate patterns predominate. No

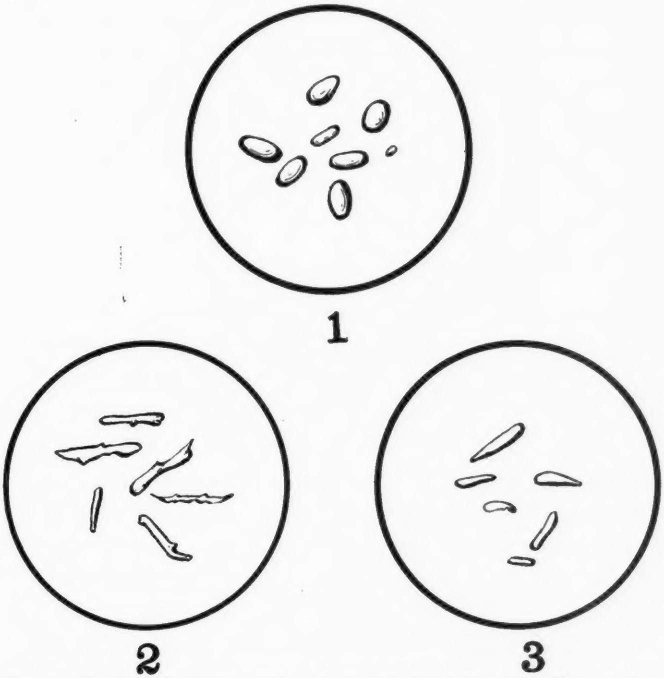

FIG. 3. Cortical pigment granules: 1, from hair of Apache Indian; 2, from median portion of yellowish wrist hair of the Squirrel Monkey (*Chrysothrix sciurea*). These were large granules, some being 1.80 microns in length; 3, from the median region of the fur hair of the American Otter (*Lutra canadensis*).

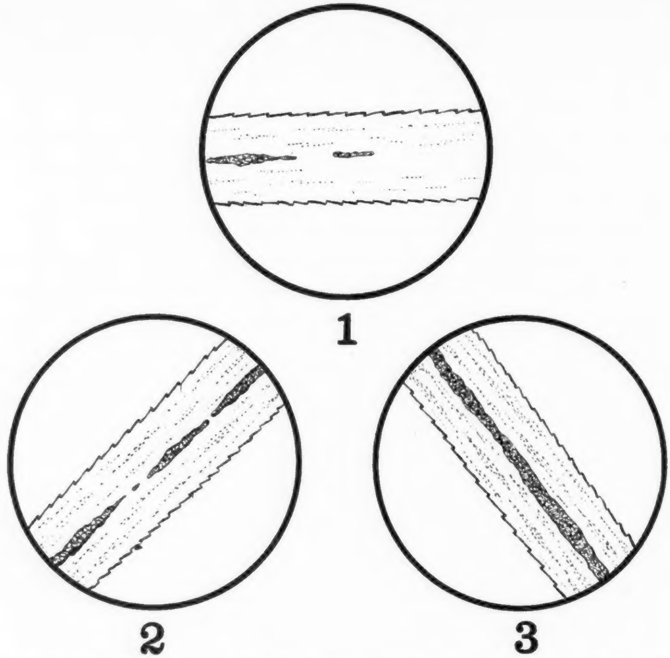

FIG. 4. Cortical pigment granule patterns in human head-hairs, showing their relationships to the colors of the hair-shafts. Each figure represents an optical longitudinal section through a hair-shaft, in the median region of the hair, along the plane of the medullary column. 1, hair of Lithuanian, Cream Buff (19″ YO–Y d); 2, hair of Somali, Benzo Brown (13‴′ OY–O i); 3, hair of predynastic Egyptian, Fuscous (13‴′ OY–O k).

correlation between the pattern-forms and the racial sources of the hairs could be found. Apparently the only correlation here was that existing between the color of the hairshaft and the shape and compaction of the granule patterns, as indicated by the graph (Fig. 5).

The melanin substance composing the pigment granules, as seen in the smallest of the granules, is of a light yellowish hue. The color of the granules deepens with their increase in size, until some appear, even by the most powerful transmitted light, jet black. Granule colors[7]

[7] The color nomenclature is that of Ridgway, in his "Color Standards and Color Nomenclature," Washington, 1912.

were found to vary between Cartridge Buff (19″ YO–Y f) and Chamois (19″ YO–Y b), through Honey Yellow, to Isabella Color (a light brown) (19″ YO–Y i); and from Light Vinaceous Cinnamon (13″ OY–O d) through all the intermediate shades given by Ridgway to Warm Sepia (13″ OY–O m), according to the diameters of the granules. The larger granules transmitted no light and appeared black, though they could be reduced to the lighter browns and yellows with nitric acid. An unsuccessful attempt was made to establish correlations between the color values and the granule diameters.

In a similar way did the color depth of the granule patterns depend upon the size of the patterns, the sizes of their component granules, and the degree of compaction of the granules.

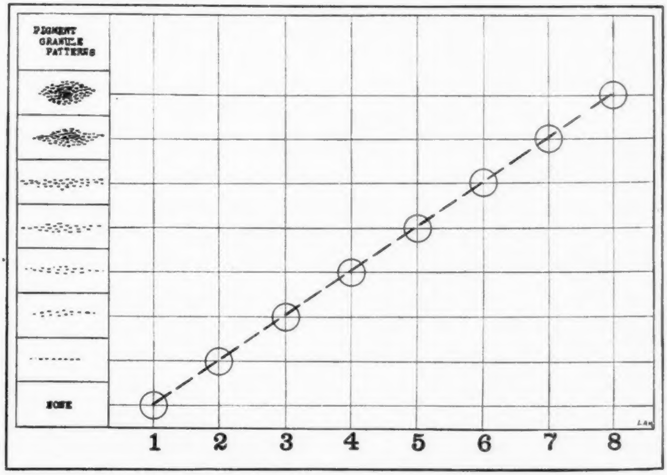

FIG. 5. The relationship of the cortical pigment granule patterns to the color of the hair-shaft (where diffuse cortical stain is absent). Hair colors (Ridgway's nomenclature[6]) are indicated by numbers, as follows: 1. White, 2. Cartridge Buff (19″ YO–Y f), 3. Cream Buff (19″ YO–Y d), 4. Isabella Color (a light brown) (19″ YO–Y i), 5. Benzo Brown (a medium brown (13‴′ OY–O i), 6. Fuscous (dark brown) (13‴′ OY–O k), 7. Fuscous Black (very dark brown, almost black) (13‴′ OY–O m), 8. Jet Black.

The various shades of yellows, through the browns, to jet black, which hairs exhibit, were produced in this way by increasing numbers of pigment granules, massing into more and more ovate patterns. These colors were modified by the presence of the diffuse cortical stain, which gave to the hairs reddish-brown tones, and coppery hues. The precise colors of these reddish-brown hairs lay in the regions of the Tawny (13′ OY–O i), the Ochraceous Tawny (15′ Y–O i), and the Cinnamon Rufous (11′ Orange i). Hair which appeared black to the unaided eye, and in a mass, under the microscope by transmitted light was seen to be merely a very dark brown, the brownish and yellowish hue of the individual granules being apparent near the cuticle of the shaft. Thus twelve samples of black hair taken from ten different racial groups (*i.e.*, Negrills (Bushmen), Bantu, Guinea, Negro, Chinese, Japanese, Mongols, Eskimo, Apache, Sioux, Cliff Dwellers) showed under the microscope at least eight appreciably different shades of brown, lying in the region of the Orange Cinnamon (13″ (OY–O) and the Buckthorn Brown (17′ O–Y i), and all produced by differing sizes and compactions of pigment granule patterns. The hairs of this series contained little diffuse pigment. All these samples appeared jet black to the unaided eye, viewed either as single hairs or *en masse*. In some specimens of black hair the granule patterns are so thickly massed together that no light can be passed through the cortex except just beneath the layer of cuticular scales.

The chief interest of the present study, however, lies not in the description of the physical characteristics of those elements producing the color of the hair-shaft, but in the fact that a study of the relationships of the colors, forms and sizes of the pigment granules and their patterns, and in the degrees of compaction of the latter as well, has revealed that the pigmentation characters of human head-hair related directly to the color of the hair-shaft, and not to racial origin. Thus brown hairs of a given hue will exhibit, under the microscope, the same

pigmentation characters, no matter what their racial source. The same holds true for the other colors occurring in human hair. Moreover in any one racial group of peoples, and sometimes upon the head, even, of a single individual, there were found hair-shafts exhibiting different pigmentation characters which the writer earlier believed were to be looked upon as indicative of different racial affinities. It must be noted in this connection that in the hairs of the Mongolian race (notable among the Chinese and Japanese) there were encountered more *elongate* granule patterns than were seen in the black hairs of some of the African tribes, chiefly the Bushmen and Bantus. These distinctions were so ill-defined, however, and admitted of so many exceptions, that they can not be regarded as having any diagnostic value.

In making the tabulations of pigment granule and granule patterns characters from which the deductions here set forth were drawn, the regions of the hair-shafts midway between the base and the tip were considered only. The hair-shaft series used for study and comparison is a permanent one, the hair-shafts being cleared in xylol and mounted in balsam. No decolorizing or softening procedures were admitted, nor any treatment which would modify the nature of the pigment granules or disturb the relation of the cells of the cortex, medulla or cuticle.

The results of this study have convinced the writer that it is virtually impossible, from a microscopic study of the hair-shaft, to determine the racial source of a hair sample, except in the broadest (and hence, for scientific purposes, unsatisfactory) way. Cuticular scales and medullas, as has already been pointed out, are not correlated with race, but with diameter of the hair-shaft.

It is now shown that pigmentation characters are correlated, not with race, but with color of the hair, and hence offer a no more usable criterion of race than was before possessed in the color of the hair as seen by the unaided eye. Moreover the pigmentation characters vary even more widely within individual and racial-group

limits than do the colors exhibited by hair *en masse*, and in this respect are even less useful diagnostically. As for identifying individuals from hair-samples under the microscope, this, it is believed is impossible, unless the hair is marked by some abnormality in its structure or pigmentation.

SUMMARY

The cortical pigment granules of human head-hair in their color or structure are related to hair-shaft color only. The patterns formed by these cortical granules, in their form, compaction, placentation and frequency in the cortex, are related to hair-shaft color only. Within racial-group limits, as well as within individual head limits, wide variations in hair-shaft structure and pigmentation occur, of such magnitudes as to warrant the supposition (if too meager an amount of material is available for study) that these variations are indicative of differences in racial sources of the samples.

A STUDY IN AMERICAN PALEOHISTOLOGY

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THE paucity of literature dealing with paleohistological studies of the ancient human remains of the two Americas is appalling as compared with that dealing with the ancient groups of the "Old World," especially Egypt. Sir Marc Amand Ruffer has made an entensive study of Egyptian and Coptic mummies dating from approximately 4000 B. C. to 500 A. D. The names of Fouquet, Flinders Petrie, Elliott Smith and Moodie are familiar to every student of Egyptology.

It was the original purpose of this paper to present in detail only the structure of the thyroid gland, but the almost complete absence of any microscopic analyses of the mummies of this continent was deemed to be sufficient reason to extend its original scope to other tissues.

For the materials for this study I am very much indebted to Dr. E. A. Hooton and Mr. S. J. Guernsey, both of the Peabody Museum of American Archeology and Ethnology of Harvard College, who have graciously allowed me to take samples from their rare collections.

The major portion of the work has been devoted to a study of the Basket Maker Indians, who are the oldest and least known group of prehistoric Americans found thus far on this continent. The age of the few individuals found is as yet not definitely known, but they antedate the better known Cliff Dwellers who are found in the same regions of Arizona from whence these bodies were taken and are thus probably not less than three thousand years old, and even a conservative estimate will place them in an age group of from 4000 B. C. to 8000 B. C. They are at least as old as most of the early Egyptian mummies and considerably older than many.

Samples were also taken from two dogs found in Basket Maker burials, and which are the oldest known domesticated dogs on this continent. Several samples were taken from Cliff Dweller Indians and from the body of a young girl found in Peru. The group thus, although small, is rather representative and covers a period in time of nearly six thousand years, as well as two remote geographic regions.

A detailed account of the culture and mode of living of these peoples would be entirely out of place here. The reader is referred to the excellent account of Mr. Guernsey, who excavated all the mummies, except Peruvian, used in this study.

GROSS APPEARANCE

The necks were taken from two Basket Maker Indians, one a male of about middle age, the other a female somewhat younger. The skin was of a dark brown color and pitted rather deeply, somewhat resembling the scars of variola. The skin is not sufficiently well preserved, however, to be able to say that the pitting is not due to sand erosion or to organisms, post mortem. The "Adam's Apple," having a cartilaginous support, is naturally quite prominent, and on either side there is a distinct ridge caused by the anterior border of the Sternocleidomastoid muscle.

No embalming fluids of any kind were used; the bodies were simply buried and then desiccated by the warm, dry sand. The peculiar drying properties of the hot sand were especially noted by Ruffer in his studies on Coptic and Greek mummies.

After softening for about twenty-four hours in a fluid which by experiment has been found best to consist of 30 cc alcohol, 20 cc 5 per cent. sodium carbonate, 5 cc 10 per cent. formalin and 50 cc water, the tissues become very soft and are easily dissected. If the material becomes too soft additional alcohol may be added, or if the tissues do not soften, more sodium carbonate may be added. The

small amount of formalin seems to help in preventing the tissues from falling apart during the process, for they become very fragile in the softening fluid. The fat of the tela subcutanea has uniformly disappeared, and the skin separates very readily from the underlying fasciae, which form an infinitely thin investment for the muscular layers. The individual muscles in the softened specimens were easily distinguished, although greatly reduced in size due to the thorough desiccation. The superficial branches of the cervical plexus were quite prominent, especially the descending supraclavicular portions. The Platysma myoides was not distinguishable, but its position could be shown by the external jugular vein.

Of the deeper neck structures the carotid sheath was one of the most markedly well preserved. The sheath was intact and much less shrunken than the muscular tissues. The descendens cervicalis hypoglossi was demonstrated on the anterior surface of the sheath, and within the sheath the common carotid artery, which measured 9 mm transversely and 3 mm anteroposteriorly. The internal jugular vein was slightly smaller than the artery, and the vagus nerve measured 1.5 mm in its greatest diameter.

The larynx and trachea were well preserved, even the vocal cords being very prominent. The laryngeal cartilages were almost entirely calcified, as would be rather expected in an individual past middle age. The esophagus could be traced for only a few centimeters in the female specimen, and was not included in the male. On the female a portion of the thorax was included, which showed calcified costal cartilages and tracheal rings. Attached to the thoracic wall (by a pleural adhesion?) was the greater part of the left lung. This fragment represented most of the lung and certainly its entire thickness, yet in the desiccated state it was only three millimeters thick. Its microscopic structure will be described later. The intercostal muscles were almost as thin as parch-

ment, yet they separated readily into their constituent external and internal layers.

The thyroid gland, which was the original objective, was the most elusive structure. It was missed on the first examination, and when found it was done so by following the superior thyroid artery to the gland. The gland when removed was nearly transparent and of a leathery texture. Each lateral lobe measured 35 mm x 22 mm x 6 mm, and the weight of the entire gland was only six grams. Both superior thyroid arteries and the thyroid veins were found, as was also the superior laryngeal nerve.

The ribs, costal cartilages, vertebrae, clavicles and their corresponding joints were examined on two Basket Maker Indians. No bony lesions of any kind were found, but the series is far too small to permit of any generalizations regarding the presence or absence of bony lesions among these peoples. The joint capsules were extremely tough, but little calcified. The intervertebral fibrocartilages were very small, tough and uncalcified. It would seem that lesions of the spinal column were less frequent among these peoples than among the early Egyptians. Bony lesions are quite common, however, in the Peruvian mummies.

In removing the vertebrae of the female body a fragment of the spinal cord consisting of four or five segments was found. The dura mater spinalis was hard and tough, but had retained its shape perfectly. Of the cord proper there remained only a small amount of a crumbly mass, which on section showed no structure whatever, not even the median septum being present.

The two dogs found with the Basket Makers were very shrunken, but externally quite well preserved. The rarity of these specimens prevented a complete study, although it was found that little remained of the viscera. Specimens of the skin and tongue only were embedded and sectioned.

A small piece of the umbilical cord was obtained from a small babe of the Basket Makers. The cord was evidently preserved as some sort of fetish, for it was tied by a string to the front part of the cradle. The baby itself was apparently a couple of months old.

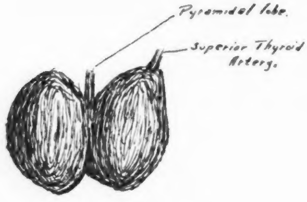
From the Cliff Dwellers were obtained pieces of trachea, lung, skin and intestine. From a young girl found in Peru samples of skin, muscle and stomach were obtained. Another Peruvian mummy yielded a sample of colon.

Microscopic Appearance

Basket Maker Indians

Skin: After embedding the skin became almost bony and it was sectioned only with the utmost difficulty. None of the tissues studied would seem to have sectioned as easily as those of Dr. Ruffer. The sections were stained with Hematoxyline and eosine, but no histological structure was evident. The epidermis was entirely absent, and of the dermis nothing was demonstrable except a formless mass of connective tissue. No nuclei of any kind were found.

Muscle: Good sections of muscle are hard to obtain, but teased preparations are rather easier to prepare and are more instructive. On preparations thus made various stains were used, such as Hematoxyline and Eosine, Picric Acid Fuchsin, Borax Carmine, Nile Blue, Niagara Blue and Neutral Red. Of these various stains Hematoxyline and Eosine gave the best results. A specimen stained with Nile Blue was the first to show what were at first thought to be artefacts, or some hitherto unrecognized gigantic microorganism. Other preparations stained with Hematoxyline and Eosine and examined under high magnification gave the appearance shown in Fig. 3. These structures are not organisms, but are perfectly preserved, although greatly reduced in size, erythrocytes. Ruffer states that blood corpuscles were never found in Egyptian mummies, and Moodie, writing on

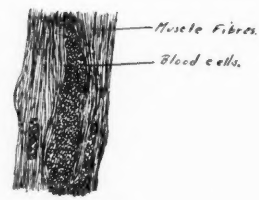


Thyroid Gland. Nat. Size.

Section of Thyroid Gland. Mag 50.

Group of Blood cells Mag 950

Mag 1700



Intramuscular Bloodvessel. Mag 500.

some possible blood cells found in dinosaur bones similar to those described by Seitz, remarks: "The studies on the Egyptian mummies have not resulted in the discovery of blood corpuscles." Schmidt examined bodies dating from one thousand years before Menes (3400 B. C.) to 500 B. C., and was unable to find a positive haemin reaction, tending to show the complete disappearance of all blood in the process of time. Wood Jones, however, is convinced that traces of blood are readily discernible. Elliot Smith has referred to blood stains on the bandages used in the primitive surgery of Egypt. Ruffer in his extensive studies into the histology of Egyptian mummies did not discover any definite corpuscles.

Friedenthal found red blood in the body of a mammoth of Pleistocene times. The precipitin reaction is similar to that of the modern elephant. No record is made of the preservation of blood corpuscles.

Inasmuch as repeated tests on Egyptian mummies had failed to reveal blood cells or positive blood reactions, there remained the possibility that these structures were particles of some foreign material that had entered the preparation. Repeated preparations, however, have proven beyond a reasonable doubt that these are red blood corpuscles that have been uniformly shrunken and dried, probably slowly and completely, for they present no signs of crenation. Under high magnification they show characteristic bi-concave surfaces, and in a few instances hundreds of them have been found in the blood vessels. (Figs. 3 and 4.) Measurements of over fifty of these cells has given an average diameter of only 1.9 microns, so they are barely one fourth the size of the present-day fresh corpuscles.

A close survey of the existing literature, part of which has been quoted above, fails to show any observations of blood cells of this antiquity being recorded, and it is interesting to note this similarity with the Egyptian mummies that, although the blood cells appear normal except for size, the usual Acetic acid-Sodium chloride test for hemin has been repeatedly negative. Hence it would appear that the haemoglobin at least has been destroyed during the processes of time, without visibly altering the corpuscles by crenation or actual disintegration.

On immersing the dry materials in the softening fluid there is immediately a dark brown viscous material extracted. This material which was thought to be blood has been recorded by nearly every man who has worked with mummy material, but its composition has remained more or less of a mystery. I am indebted to Dr. Fiske, of the department of biochemistry of the Harvard Medical School, for making tests on this material for me. Haemoglobin tests are entirely negative, but tests for iron showed it to be present in a concentration of approximately two one-hundredths of a milligram per cubic centimeter. This is an infinitely small amount of iron, but only analyzed materials were used in the preparation of

the softening fluids and so the amount present bears some relation to that present in blood, and as the amount was less in skin and more in lung, where more blood would be expected, it hardly seems that this iron can be of extraneous or post-mortem origin.

In one or two preparations only have nuclei been observed. These probably belong to the connective tissue rather than to the muscle. Their very rarity throws considerable doubt on them, and the possibility of their being artefacts can not be overlooked. The cross striations of voluntary or skeletal muscle are usually seen and are more easily found in teased and unstained preparations.

Blood vessels: Sections of both the common carotid artery and the internal jugular vein show no Tunica intima whatever. The Tunica media is separable, but neither it nor the Tunica adventitia show histological structure.

In spite of the few structures remaining histologically structural or differentiated it is interesting to see that which remains, for these bodies were never in any way preserved or embalmed, except by the action of nature through the medium of warm sand and an unusually dry climate. Yet these bodies show almost the same histological differentiation observed on the Egyptian mummies that had been so carefully preserved both by embalming and by wrapping. The Coptic mummies mentioned by Ruffer were simply sand dried and lost most of their microscopic structure in a trifle over fifteen hundred years.

Thyroid gland: In gross appearance after softening, the gland was nearly transparent, of a dark brown color, and somewhat leathery to the touch. On section and examination nothing remains except the connective tissue of the lobules. (Figs. 1 and 2.) No epithelium is present, as would be indicated by the weight of the gland as compared to its size, it having a weight of only six grams and a volume of nearly four thousand five hundred cubic millimeters. The sections look much like sponge, and the remaining connective tissue shows that the lobules were

essentially of about the same size as are found to-day in the normal or slightly adenomatous thyroid gland. Whether the apparent increase in size of the lobules is due to the action of the softening fluids or to ante-mortem pathology is a matter for speculation only. Unfortunately these people left no records that might throw any light on the matter.

Lung: The most striking feature of this organ is its extreme thinness and the immense quantity of a carbonaceous or silicious material present throughout. This material, which is apparently a mixture of soot and fine sand or dust, is present throughout the lung and simulates exactly the condition known to-day as Silicosis. This was apparently a common condition among this group, who lived in a dry and dusty atmosphere, and may offer an explanation for the pleural adhesion referred to before. Undoubtedly much of the material is extraneous and post-mortem, but that in the alveoli must have been present during life. The alveoli are greatly compressed, and only an occasional bronchiole has remained. Otherwise the lung is nearly structureless. A few sections stained with Resorcin-Fuchsin have shown the presence of a few elastic connective tissue fibers.

Umbilical cord: This fragment was very small and hard, and when broken the broken surfaces had a glistening metallic-like luster. In the softening fluid it gave off a large quantity of the aforementioned brown fluid which was positive to microchemical iron tests. On section only a part of one wall remained, including one of the umbilical arteries and a mass of spongy material that has the appearance of the well-known Wharton's jelly. No blood cells were found here.

Cliff Dweller Specimens

Inasmuch as the material is from the same region and although of a much later period (approximately 2000 B. C.), it is so similar to the foregoing Basket Maker Indians that it will be dismissed very briefly.

The trachea, lung and skin were almost identical with those taken from the earlier peoples. The lungs showed Silicosis, and with Resorcin-Fuchsin showed elastic connective tissue. A few blood corpuscles were found in sections of the skin, and there was considerable pigment present throughout. There was no mucosa nor serosa on samples of intestine.

Peruvian Mummy

The skin of this young girl was much lighter than that of the North American peoples and was better preserved. The age of this mummy is not certain, but it would apparently belong to a period of about a thousand years ago, or nearly the same age as the Coptic mummies of Egypt. No epidermis was present nor were nuclei found at all in the skin. The stomach showed faint separation into its component muscular levels, and numerous blood corpuscles were found in the walls. No nuclei were found in the stomach musculature, in the skeletal muscles nor in the colon. There was also an absence of the colon's mucosa and serosa.

Dogs

The larger of the two dogs is about the size of the present-day Spitz, but with a more ferocious expression. The hair is very well preserved, dry but rather soft, and of a mottled light brown and white. On section the hairs seem little changed except for the absorption of the medulla. The hair follicles present little but the connective tissue. The epidermis is wanting, though the remaining layers of the skin are distinguishable.

A section of tongue from this dog is more characteristic than the other structures, and what was apparently the sub-lingual salivary gland can be readily recognized from the well-preserved connective tissue of the lobules. The lingual papillae are not prominent because of the distortion caused by drying.

The smaller dog is more of a wild type, and black with a few white spots. The sections of skin, except for the

hair color, are the same as those of the white dog. The tongue was much smaller and consequently less well preserved. The muscle fibers of it can be identified but the mucosa is gone.

Conclusions

Blood corpuscles are herein reported for the first time in tissue from human mummies of the American continent approximately six to ten thousand years old. The preservation of these ordinarily delicate structures, while nuclear structures have disappeared, can not at this time be given a satisfactory explanation.

Evidences of pathology in this small group are few, but it is hoped that a more extensive survey will bring to light more pathology, and perhaps more bodies will bring to light better histological structure.

Summary

(1) The external characteristics of the human body are well preserved in unembalmed bodies buried in warm sand at least three or four thousand years ago.

(2) The fasciae, nerves and blood vessels are well preserved in these bodies.

(3) The muscles are greatly shrunken and show no nuclei, but show cross striations, which are best seen in teased and unstained preparations.

(4) Blood corpuscles measuring 1.9 microns in diameter are reported for the first time in these ancient tissues. Hemoglobin tests are negative, but iron tests are positive.

(5) A condition of Silicosis of the lung with pleural adhesion was found.

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SHORTER ARTICLES AND DISCUSSION

OCCURRENCE OF THE MARINE CRAB, *CALLINECTES ORNATUS*, IN BRACKISH AND FRESH WATER

It is well known that the common blue crab of the Atlantic coast of America frequently migrates into brackish or even into almost fresh water, although it is typically a marine species. That this form, *Callinectes sapidus*, is able at least to survive for a considerable length of time in water of greatly reduced salinity is shown by many records of its occurrence in brackish inlets, tidal rivers and even in ponds bordering such places. Hay ('04) in his "Life History of the Blue Crab" cites a number of such instances. He observed the species in nearly fresh water of ponds and ditches often at distances of a mile or two from the shore along the eastern and western borders of Chesapeake Bay, and says that it occurs in the Potomac River and Eastern Branch opposite the city of Washington. There are several specimens in the U. S. National Museum collected by Professor S. F. Baird at Ossining, N. Y., some thirty-five miles above the mouth of the Hudson River and a specimen has been recorded from Newburgh, N. Y., twenty miles further up the Hudson. Churchill ('17) who later published an extensive account of the blue crab cites the occurrence of numerous examples in water fresh enough to drink in Back Bay at the extreme southeastern tip of Virginia south of Chesapeake Bay. *Callinectes sapidus* occurs very rarely in the West Indies, but Miss Rathbun ('95) cites one example obtained by Dr. R. P. Bigelow in Jamaica in fresh water at the mouth of the Rio Cobre. It appears then that the species tends to enter fresh water in the tropics also. Along the southern Atlantic states, the Gulf of Mexico, Caribbean Sea and Atlantic coast of Brazil there occurs a variety known as *C. sapidus acutidens* Rathbun, and this has once been reported from the brackish water of the St. Johns River at Palatka, Florida.

During a recent stay at the Harvard Biological Station at Soledad, near Cienfuegos, Cuba, I noticed a species of Callinectes in the Rio Caonao at Belmonte, seven miles above the point where the river enters Cienfuegos Bay. The tides penetrate to beyond this point, and a small sample of water collected from the river proved to be decidedly brackish, showing a density about

one fourth that of sea water.[1] At this point the river is lined by small mangroves, which give evidence of its brackish condition. Further up the river and connected with it at seasons of high water, but then separated by a mile of dry land which was last flooded by the autumn hurricane several months before, we found another large male callinectes in a small pond of quite fresh water. This individual, which proved to be *Callinectes ornatus* Ordway, was perfectly active and happy and quite belligerent when removed from the water where he had been living for at least three months. How much longer he may have been there is a matter of conjecture, but as the Rio Canao is said to have risen fifteen feet from rains at the time of the recent hurricane it seems probable that his migration may have occurred then.

I have been unable to find any reference to the occurrence of *C. ornatus,* nor of any of the several other species of Callinectes away from salt water. Certainly *ornatus* is as easily acclimated to fresh water as the well-known *sapidus,* and when the other species have been more carefully observed it is probable that they will be found to be likewise tolerant to water of greatly decreased saline content. Whether this tolerance may be associated with a decreased content of sodium chloride in the blood I do not know, but it is interesting to note that Duval ('24) has found the blood of the European *Portunus puber,* a swimming crab of the same family (*Portunidæ*), to contain considerably lower concentration of sodium chloride than that of *Carcinus mœnas.*

Most interesting studies relating to the occurrence of marine Decapoda are contained in the "Fauna of Chilka Lake" (Kemp, '15), an estuarine lake in India. Nearly sixty species, including three species of Portunidæ, are known to inhabit this lake, of which thirty-six are able to live under the extreme range of salinity of 1.000–1.0265 which has been observed there. Other species are clearly unable to withstand either the higher or lower salinities, which vary in location and shift with the procession of the seasons from highly saline in the dry season to practically fresh in the season of heavy rains.

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Y-CHROMOSOME INHERITANCE—A REVIEW

The theory that the chromosomes are the vehicle of heredity has been established chiefly from the study of Drosophila, but there is in Drosophila one chromosome which until recently has been thought not to be a carrier of genes. This is the so-called Y-chromosome, normally present in males alone. Since the males have one X- and one Y-, whereas the females have two X-chromosomes and no Y, it follows that if genes were present on the Y, they would be transmitted only in the male line of descent, never to or through a female.

The first evidence that in any organism transmission occurs requiring a Y-chromosome for its vehicle was produced by Johan Schmidt (1920). In the aquarium fish Lebistes he showed that male offspring, as regards certain color characters, always resemble the male parent. Winge (1927a) has extended the investigation and describes eighteen sex-linked characters. There are nine genes located on the Y-chromosome, three on the X-chromosome, and five or six that may cross over between the X and the Y.

Aida (1921) was the first to report crossing over between an X- and a Y-chromosome, his observations being made on the body color of the fresh water fish Aplocheilus and showing the crossing over to occur only in male parents.

In 1922 Castle published an interpretation of the Schofield family tree, indicating that webbed toes in man may be inherited on the Y-chromosome.

Goldschmidt (1923) suggested that in the moth Lymantria the Y must carry a determiner for femaleness, since in moths, as in birds, the males are XX and the females XY. Goldschmidt's Y does not correspond exactly with the Y discussed by the other authors cited, since they describe a chromosome peculiar to males, whereas Goldschmidt applies the term to a chromosome peculiar to females.

In 1925 Zulueta made the discovery of Y-chromosome inheritance of elytra-coloring in the beetle Phytodecta.

Winge (1927b) makes claim to the first authentic demonstration of this mode of inheritance in the plant kingdom. *Melandrium album* exhibits a recessive male-linked character "chlorina." Since two autosome genes are epistatic to chlorina, it can only appear in their absence, and only in male plants.

A significant paper, extending Y-chromosome inheritance to Drosophila, has just been published by Curt Stern (1927). Extensive evidence is adduced to support his conclusion that the Y-chromosome of *Drosophila melanogaster* carries the inhibitor or normal allelomorph of the character "kurzborstig" (bobbed, symbol bb.). As described by Mohr (1924) bobbed is a sex-limited recessive, situated on the first chromosome, locus 70. In homozygous females this gene is expressed as a shortening of bristles on the head and thorax accompanied by pronounced abnormalities of the abdominal bands. The males of *D. melanogaster* do not exhibit this character, although it is apparent in *D. simulans* males, whenever the gene is present.

Breeding tests proved that the inhibitor was not linked with bar on the first chromosome, nor with curly, hairless or eyeless, on the second, third and fourth chromosomes, respectively.

Non-disjunction females (XXY), although homozygous for bobbed, failed to show the character. On the other hand, the exceptional (XO) males were markedly bobbed.

Stern also observed four gynandromorphs, each homozygous for bobbed. Three of these were visibly bobbed on the female side, as one would expect, but, also, on the male side. The tissue of this region was probably of the XO constitution, and so lacked the inhibitor normally carried in the Y-chromosome. A fourth specimen was one quarter female and bobbed, and three quarters

male and normal. This condition was interpreted as due to the presence of a Y-chromosome in the normal (male) tissue. Apparently two zygotes had fused, an XX and an XY zygote, to result in such a gynandromorph.

The evidence is rounded out by a cytological study. Phenotypically bobbed females were shown to possess the normal equipment (XX), whereas ten exceptional females in which the bobbed genes were inhibited were shown to possess an extra Y-chromosome (thus being XXY).

Stern's very extensive evidence indicates that the Y-chromosome of *Drosophila melanogaster* may be responsible for the sex-limited behavior of a specific character. Now that the Y-chromosome has been aligned with the others in confirmation of the chromosome theory of inheritance, one awaits with interest the demonstration of a character limited to the female sex, corresponding to the sex limited (or one-sided) inheritance of a W-chromosome in birds and moths. Since Y-chromosome inheritance has been demonstrated in Drosophila, Coleoptera, Vertebrata and in Melandrium, this phenomenon may be considered to be of widespread occurrence in the plant and animal kingdoms.

R. C. Robb

Bussey Institution

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THE CHROMOSOMES OF RANUNCULUS ACRIS

Because of the wide variability in somatic characters, which has long been observed in *Ranunculus acris,* and because of the variation in chromosome complex which has been recently found, considerable attention is now being given to the cytology of *Ranunculus acris.* Of special interest is the fact that together with the plants which have a very constant number and morphological distinction of the individual chromosomes, strains are sometimes found in which the chromosome number deviates from the normal. The purpose of this paper is to bring together the results on cytology of *Ranunculus acris* obtained by three authors on material from entirely separated regions, namely the work of Sorokin (1924),[1] Senjaninova (1926),[2] Sorokin (1927a),[3]

[1] Sorokin, Helen, "The satellites of the somatic mitoses in *Ranunculus acris* L." Publ. Fac. Sci. Univ. Charles (Prague) 13: 1–15. 1924.

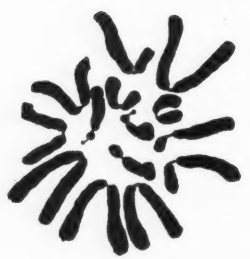
[2] Senjaninova, M., "Das Verhalten des Nucleolus und der Tarabanten während der somatischen Mitosen und den Reifeteilungen bei Ranunculus acer L." Zeitschr. Zellforsch. u. mik. Anat. 3: 417–430. 1926.

[3] Sorokin, Helen, "A Study of Meiosis in *Ranunculus acris.*" *Amer. Jour. Bot.* 14: 76–84. 1927.

(1927b),[4] and Langlet (1927).[5]

In 1924 Sorokin described a normal form of *Ranunculus acris* with 12 chromosomes, a gynodimorphic form with 18 chromosomes and plants with characters intermediate between the normal and the gynodimorphic with 13, 14 and 15 chromosomes. In 1925 a normal form with 14 chromosomes and gynodimorphic plants with other chromosome numbers than 18 have been found by the writer.[6] Further studies showed that the 14-chromosome race of *Ranunculus acris* is the most common, and that it has the following somatic chromosome formula: $2A + 2B + 2C + 2c + 2d'' + 2e + 2f$ (fig. 8).

1 2 3 4 5 6 7



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All the chromosomes in *Ranunculus acris* differ from one another in size, shape, place of constrictions, and two chromosomes are further distinguished by the presence of the satellites. The A-chromosomes are V-shaped with median constriction, each of the arms being 5.1 microns in length (fig. 1). The B-chromosomes have one arm 4.8 microns in length, and another arm 1.7 microns; a very distinct constriction is found between the two arms (fig. 2). The C-chromosomes are V-shaped, both of the

[4] Sorokin, Helen, "Cytological and Morphological Investigations on Gynodimorphic and Normal Forms of *Ranunculus acris* L." *Genetics* 12: 59–83. 1927.

[5] Langlet, O. F. I., "Beiträge zur Zytologie der Ranunculazeen." *Svensk Bot. Tidskrift* 21: 1–17. 1927.

[6] See Harris, J. Arthur. Activities of the Department of Botany, University of Minnesota, 1925, pp. 23–24.

arms being 4.7 microns in length. A pronounced constriction is found between the two arms (fig. 3). These chromosomes were not observed in the 12-chromosome race of *Ranunculus acris* studied previously. The c-chromosomes are V-shaped with median constriction, both of the arms being 3.4 microns in length (fig. 4). The d-chromosomes are rod-shaped with a distinct constriction at one end and with a small satellite attached to the constricted part of each of the d-chromosomes. The long part of the d-chromosomes is 4 microns in length, the constricted part is 0.4, and the satellite is immeasurably small (ε) (fig. 5). The e-chromosomes are rod-shaped with very pronounced terminal constriction. The long part of the e-chromosomes is 4.3 microns in length, while the constricted part is 0.9 (fig. 6). In certain metaphasic plates the e-chromosomes are often arranged in such a way that the constriction is not visible, these chromosomes therefore often appear as if hook-shaped. The f-chromosomes are definitely constricted and consist of two arms, one arm being 2.9 microns in length and another arm 1.7 (fig. 7). These chromosomes are always found in the center of the metaphasic figures and have been previously referred to as rod-shaped. Replacing the letters in the somatic formula by the numerical data obtained from the dimensions of the chromosomes in microns we will have total length of the 14 chromosomes of *Ranunculus acris*: $2(5.1+5.1)+2(4.8+1.7)+2(4.7+4.7)+2(3.4+3.4)+2(4.0+0.4+\varepsilon)+2(4.3+0.9)+2(2.9+1.7)=94.2+2\varepsilon$.

In previous work (Sorokin 1927b) somatic formulae have been used to indicate the morphological distinctions of the chromosomes in *Ranunculus acris.* The large chromosomes were indicated by capital letters. In the present study actual measurement of the chromosomes showed that the large chromosomes A, B, and C have arms (both or only one) ranging from 4.7–5.1 microns, the medium chromosomes c, d, e have arms ranging from 3.4–4.3 and the small chromosomes have arms shorter than 3 microns.

Senjaninova (1926) described 14 somatic chromosomes in *Ranunculus acris.* Two chromosomes were found to be rod-shaped, twelve chromosomes with two arms. In six chromosomes both arms were of equal length, and in six remaining chromosomes one of the arms was one half the length of the other. Senjaninova did not note, however, that the chromosomes differ in size and did not indicate the presence of definite constrictions. She reported also a race of *Ranunculus acris* which in external

morphology was indistinguishable from the normal but which had 29–32 chromosomes. In this race she found twelve chromosomes with two equal arms, twelve chromosomes with arms of unequal length and four rod-shaped chromosomes with satellites. Thus besides the tetraploid chromosome complement of 28 there were found some additional chromosomes the exact nature of which has not been determined.

In a recently published paper Langlet (1927) described 14 chromosomes as the diploid number for the normal form of *Ranunculus acris.* The same number of chromosomes is given for a form in which in spite of occurrence of more or less regular reduction division the stamens became shriveled. An interesting case of the increase in the number of the chromosomes is also described. In addition to the regular 14 chromosomes in all the metaphases of a root tip of a normal *Ranunculus acris* Langlet observed some extra chromosomes. These chromosomes were short, all of the same length and as thick as the normal chromosomes. The number of the extra chromosomes varied from two to ten, six being the most frequent. The distribution of the extra chromosomes was as follows:

Number of extra chromosomes	2	3	4	5	6	7	8	9	10
Number of metaphasic plates	1	1	21	40	148	23	14	1	3

Langlet suggested that the extra chromosomes might have originated as a result of segmentation of one or several chromosomes, and as an evidence he cites the constriction which occurs at the end of one chromosome in his figure. We can not fully agree with the interpretation of Langlet. As indicated above the chromosomes in *Ranunculus acris* all possess definite morphological distinctions, and the constriction mentioned by Langlet represents apparently either the short arm of one of the B-chromosomes, or the constricted part of the e-chromosomes. In both cases this constricted part represents a constant morphological feature of the idiogram of *Ranunculus acris.*

In a study of aberrant races of *Ranunculus acris* (Sorokin 1927b) plants with additional chromosomes were found. Medium and small chromosomes were only involved in variation. Crosses between different polyploid form and segregations taking place in the subsequent generations were proposed to be the source of polymorphism in *Ranunculus acris,* as well as the source of the occurrence of the extra chromosomes.

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Devoted to the Advancement of the Biological Sciences with Special Reference to the Factors of Evolution

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